

Progenesis Q1 Direct Sample Analysis User Guide

**Analysis workflow guidelines and
demonstration**

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Contents:

| | |
|--|-----------|
| Introduction..... | 3 |
| Important Notes | 4 |
| Demonstration Runs..... | 4 |
| Licensing the Demonstration Runs..... | 4 |
| Create an Experiment | 5 |
| Import the Data | 8 |
| Carry Out Automatic Processing..... | 10 |
| Review Processing | 13 |
| Initial Statistical Analysis | 17 |
| OPLS-DA Statistical Analysis Using EZinfo | 21 |
| Compound Identification..... | 32 |

Introduction

Waters Direct Sample Analysis technologies are a combination of high performance mass spectrometry and innovative direct sampling tools¹.

A key example is the REIMS (Rapid Evaporative Ionisation Mass Spectrometry) Research System, with its innovative iKnife hand-held sampling device^{2,3}. This technology allows the rapid analysis of small molecules derived from a sample without any extensive sample preparation or chromatography being required (*Figure 1*). REIMS is the subject of this guide.

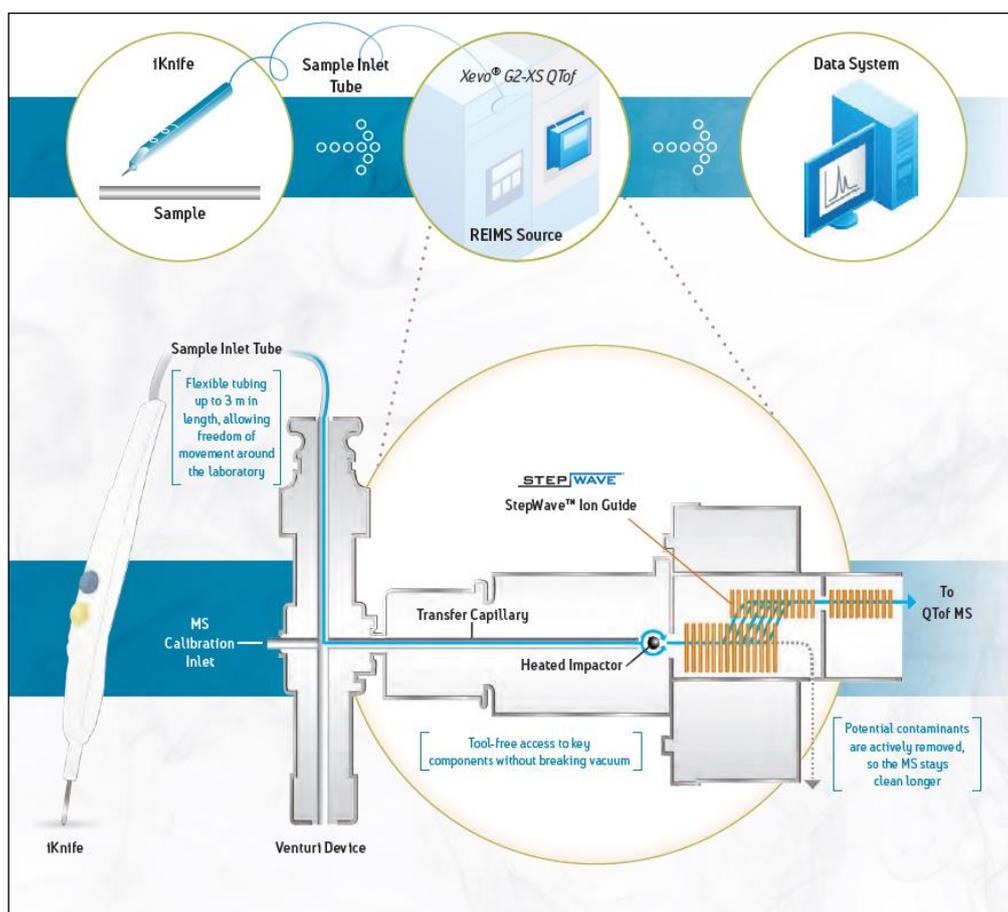


Figure 1: Schematic of REIMS iKnife-based Direct Sample Analysis.

Immediate generation of sample data with minimal processing carries many advantages. On the other hand, the lack of sample preparation, fractionation or chromatographic separation could present analytical challenges to the user without dedicated software solutions.

Such a solution has been developed for the analysis of small molecules by REIMS Direct Sample Analysis in Progenesis QI v2.1. This workflow allows a user to visualise, quantitate, discriminate and identify sample metabolites. All that is required is that the user applies Progenesis Bridge (included with MassLynx) to the analysis output files, and then selects the appropriate workflow option in the Progenesis QI software.

Progenesis QI cannot process Direct Sample Analysis *imaging* data, such as MALDI (Matrix-Assisted Laser Desorption/Ionisation) and DESI (Desorption Electrospray Ionisation) imaging⁴. However, DESI ionisation in a *non-imaging* mode produces similar data to REIMS, and could be analysed using Progenesis QI.

References:

1. Direct Sample Analysis. URL: http://www.waters.com/waters/en_US/Direct-Sample-Analysis/nav.htm?cid=134848674
2. REIMS Research System with iKnife for Direct Sampling. URL: http://www.waters.com/waters/en_US/REIMS-Research-System-with-iKnife-for-direct-sampling/nav.htm?cid=134846529
3. REIMS Research System with iKnife Sampling brochure, part number 720005418en. Available from URL: <http://www.waters.com/waters/library.htm?lid=134846772>
4. Mass Spectrometry Imaging. URL: http://www.waters.com/waters/en_US/Mass-Spectrometry-Imaging-Technologies-including-MALDI%2C-DESI-and-Ion-Mobility/nav.htm?cid=134833481

Important Notes

This user guide is a specific quick-start guide to using the **Direct Sample Analysis** workflow, covering all the basic analysis steps and options available to you. If you wish to know more about the use of Progenesis QI for LC-MS-based small molecule analysis, please consult the full user guide, which is freely available online:

<http://www.nonlinear.com/progenesis/qi/v2.1/user-guide/>

If you wish to understand the general quantitation and identification approaches in Progenesis QI in more detail, please consult the detailed online FAQ guidance:

<http://www.nonlinear.com/progenesis/qi/v2.1/faq/>

Demonstration Runs

The data used to demonstrate the workflow in this quick-start guide are REIMS burns from different fish species (a speciation study). These are available as files ready-processed with Progenesis Bridge. However, you can easily follow this guide through with your own bridged data if you prefer.

The data should be extracted from **Progenesis QI DSA Data.zip**. They are divided up into two folders. The first, **Main Demonstration Data**, contains the 25 raw files necessary to follow the main flow of the demonstration, along with a ready-made experiment design file that will help you set up the relevant experimental groupings for them. The second, **New Sample**, contains one further 'unknown' run which allows the demonstration of the [speciation of a new sample](#) later in the document.

Licensing the Demonstration Runs

If you are following the guide through with the demonstration data provided, and you do not already have a Progenesis QI license, please note that you will firstly need to license the files for use. This can be done by extracting the file **RunLicense.ief** which is provided with the data in the same archive. Copy this file to the desktop of your analysis computer, and double-click it. The images will now be usable.

Create an Experiment

The first step in Progenesis QI is to create the experiment – a project within which you can carry out the analysis of your data. You will need some basic information about your experiment.

To create a new Direct Sample Analysis experiment:

1. Load Progenesis QI v2.1.
2. Below **Other experiments** at the welcome screen, click the **New** button ([Figure 2](#)).
3. Name your experiment appropriately in the pop-up dialog box, and choose where you wish to save the results of your analysis as you progress ([Figure 2](#)).
4. Click **Next**.
5. In the following dialog, use the drop-down menu to select **Direct sample analysis** as your machine type. Specify whether you have profile or centroided data, and the correct ionisation polarity for your analysis ([Figure 3](#)).

For the example, the data are profile mode, and of negative ionisation polarity.

6. Click **Next**.
7. In the next dialog box, select adducts appropriate to your ionisation method ([Figure 4](#)). At the identification stage of the workflow, these adducts will be considered as potentially being present.

For the example, only [M-H] is defined.

8. Click **Create Experiment**. This will take you to the **Import Data** page.

Create an Experiment – figures:

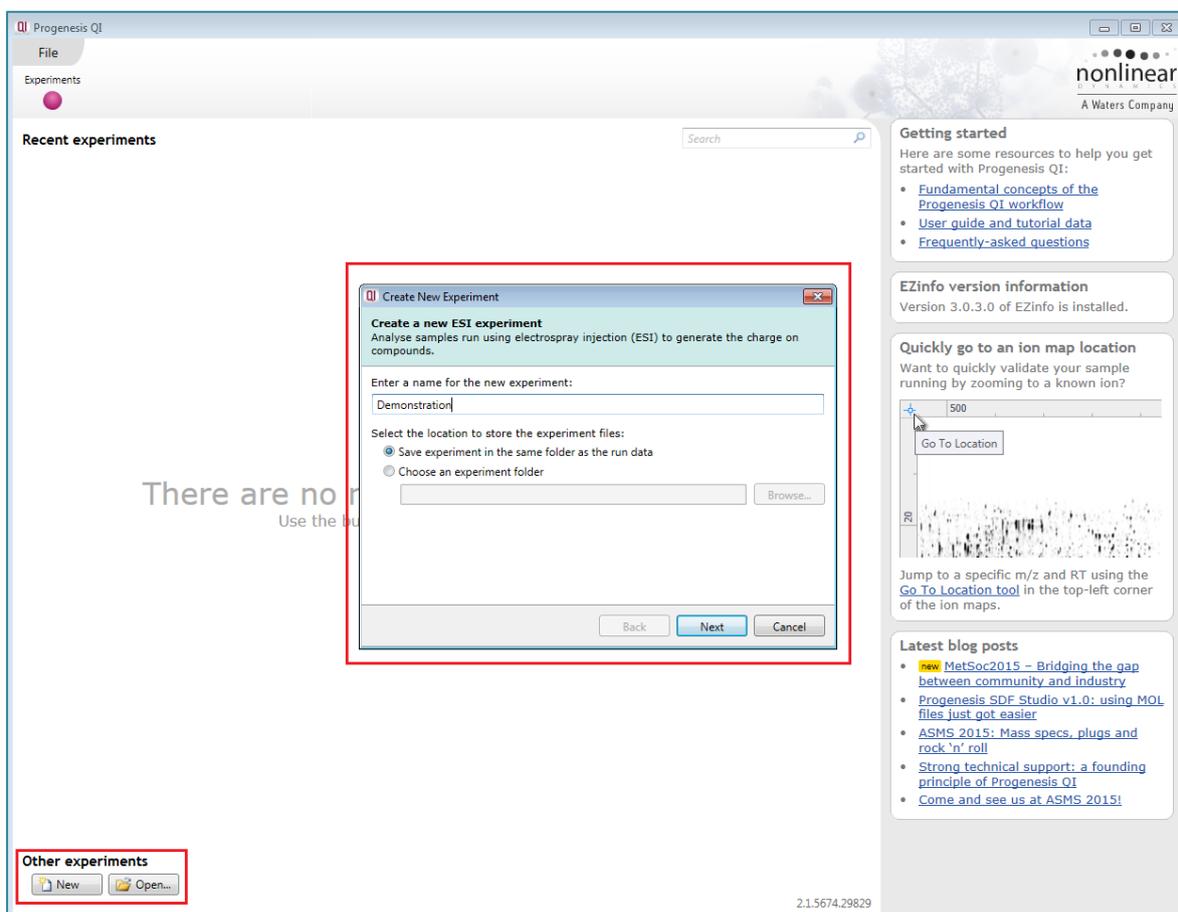


Figure 2: Creating a new experiment – generation and naming.

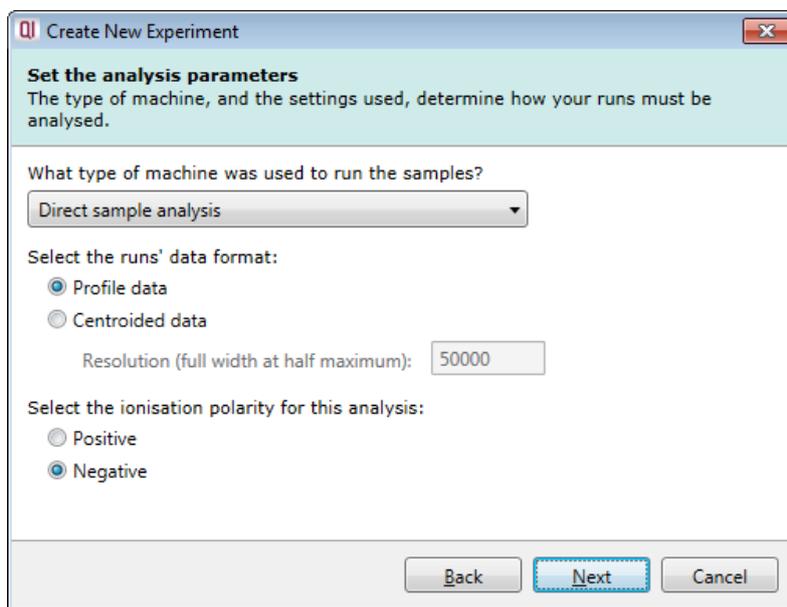


Figure 3: Creating a new experiment – selecting the Direct Sample Analysis method, data format and ionisation polarity. For the demonstration data, the data are in profile mode, and the polarity is negative.

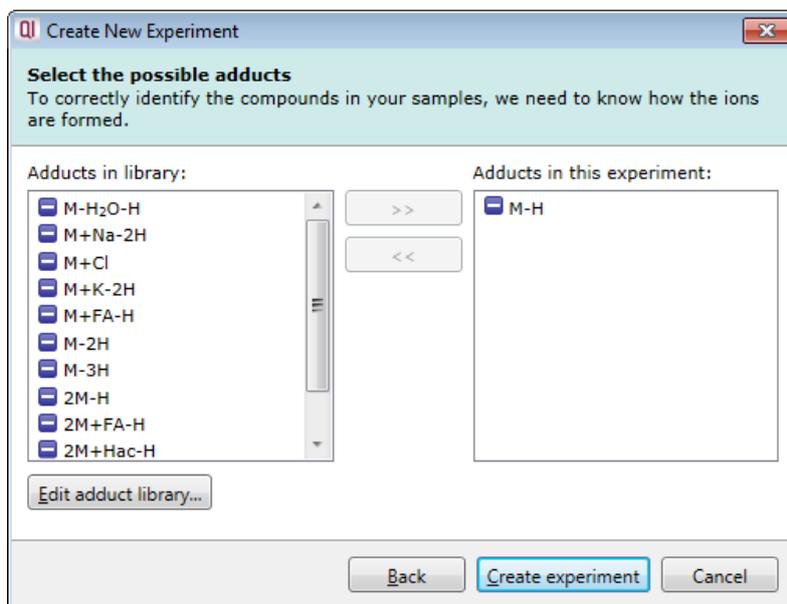


Figure 4: Creating a new experiment – defining the adducts that may be present in the experiment. For the demonstration data, M-H is selected.

Import the Data

The next step is to import your Direct Sample Analysis data. Some pre-processing should have already been carried out, as detailed below. For the demonstration data, this has already been done.

Progenesis Bridge:

Raw data files will initially be acquired and visualised using Waters' MassLynx software, and may consist of several 'burns' in the case of REIMS data, where the user has performed several samplings in the same acquisition.

These data are only resolved within a 'burn' in the m/z dimension, without any chromatographic separation. To correctly handle this type of data in Progenesis QI, a tool called **Progenesis Bridge** is used to convert the MassLynx raw data for analysis. This tool performs background subtraction, lockmass correction, the creation of extracted ion chromatograms for each burn and separation of multiple burns in each raw folder into individual raw folders. This also allows them to be correctly treated as individual samples.

Once this process has been performed, the raw data are ready for importing into Progenesis QI, where they can be visualised, quantitated and statistically classified, and discriminating small molecules can be identified.

Progenesis Bridge is supplied and supported separately, being included with MassLynx.

Summary:

- ➔ This guide assumes that you already have bridged data available in the Waters .RAW format.

Importing bridged data into Progenesis QI:

1. At the **Import Data** page, select the **Waters (.raw)** format ([Figure 5](#)).
2. Click on **Import**.
3. In the pop-up dialog, navigate to and select the folder that the raw files are contained within ([Figure 5](#)).
4. Select all the files you wish to analyse.
5. Click on **Next**. The software will examine the folders, and inform you of their readiness for import ([Figure 6](#)). Note that lock mass calibration is performed by Progenesis Bridge, and so there is no *further* lock mass calibration performed at this stage, which the software recognises.

For the example data, there should be [25 files](#) in total.

6. Click on **Import**.

You may set up and start the automatic processing in the next section while runs are importing, or wait till import is complete.

Import the Data – figures:

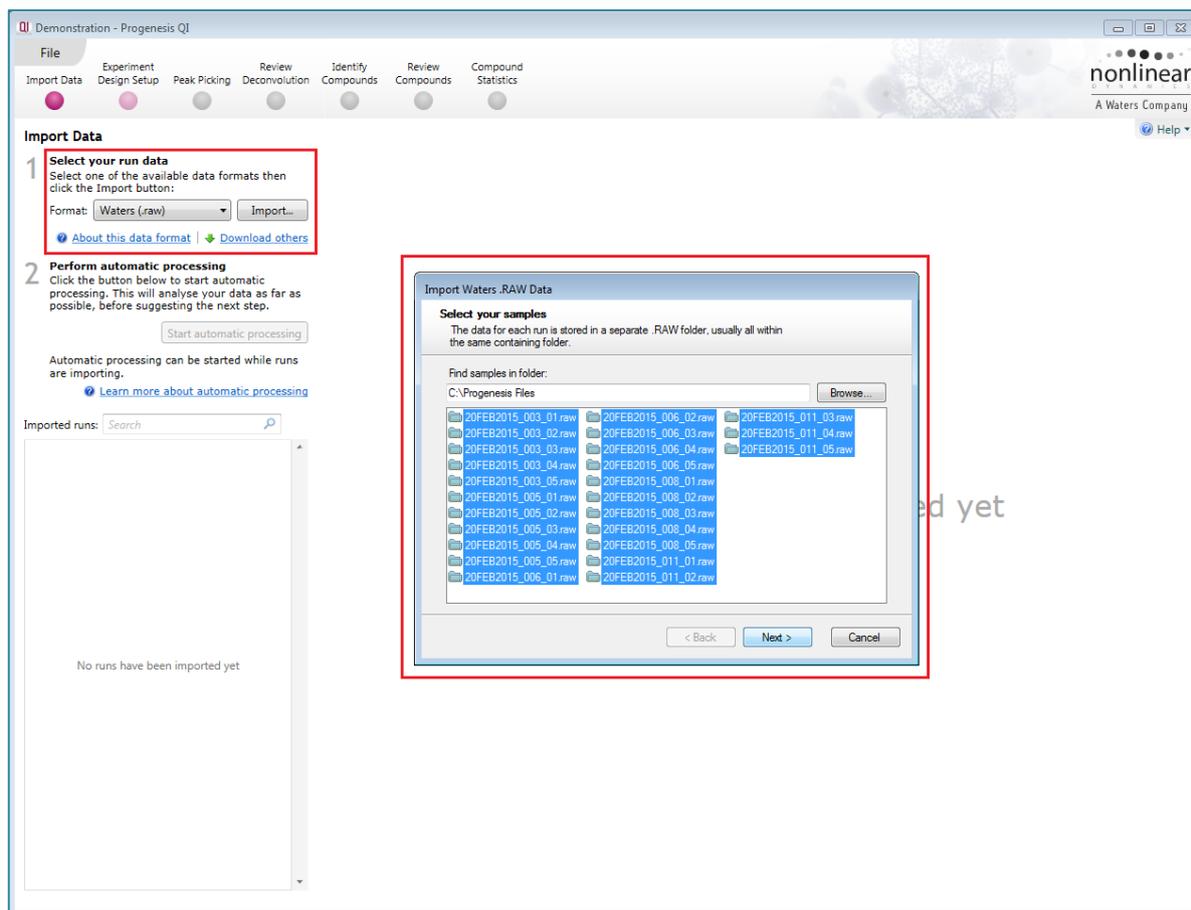


Figure 5: Selecting the correct file format, and the samples to be imported. Each sample is one 'burn' from an original multi-burn acquisition.

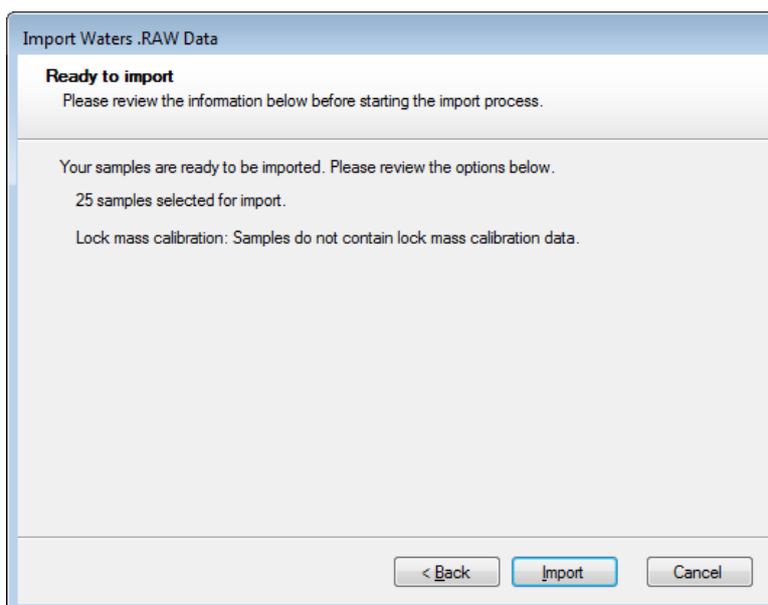


Figure 6: Reviewing the sample information prior to import.

Carry Out Automatic Processing

Once the data have been imported, the software allows you to automatically detect and quantitate the ions present, and carry out statistical analyses upon the results. The relevant steps can also be carried out individually, but using the automatic processing option is usually the quickest and most convenient way to analyse your data.

For more information on each screen, please consult the main user guide and online FAQs, as described in the [Important Notes](#).

1. Once your runs have imported, or while the import is occurring, select **Start automatic processing** ([Figure 7](#)).
2. A dialog box will appear marked **Experiment design**.
3. If you wish, define an experimental design for discriminating between your sample groups that the 'burns' are derived from ([Figure 8](#)). If you do not create a grouping now, you can do so later.

For the example data, the file **Fish Groups.csv** can be used to automatically carry out this grouping for you.

4. Click **Next**.
5. **Peak picking** will be displayed. This should be carried out, and so the box should remain checked. The parameters can be adjusted, but should not be, as they are already optimised for Direct Sample Analysis detection ([Figures 9 and 10](#)).
6. Click **Finish**.
7. Auto-processing will now occur, and the software will inform you of the results ([Figure 11](#)). If your experiment is still importing runs, then the auto-processing will proceed on completion of import.
8. Click **Close**.

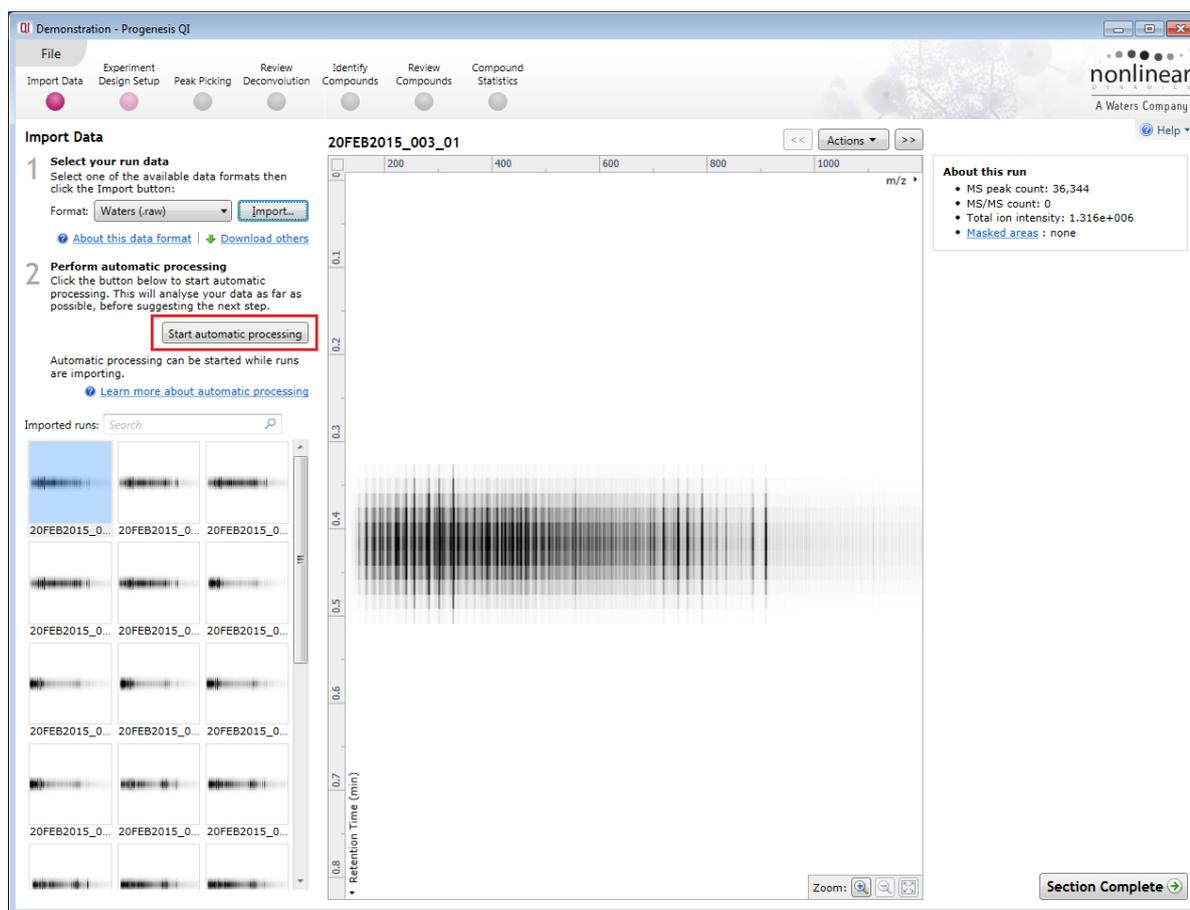
Carry Out Automatic Processing – figures:

Figure 7: Completed import, with the **Start automatic processing** button highlighted.

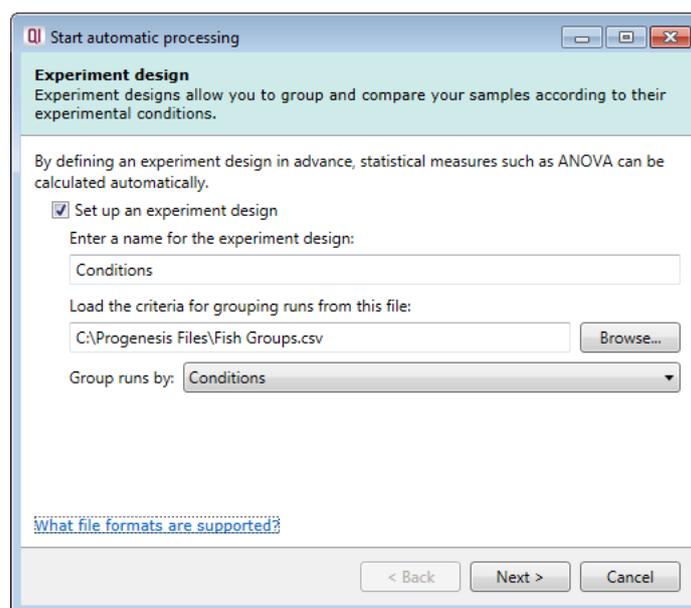


Figure 8: Selecting an experiment design file (in this case, the csv file supplied with the demonstration set).

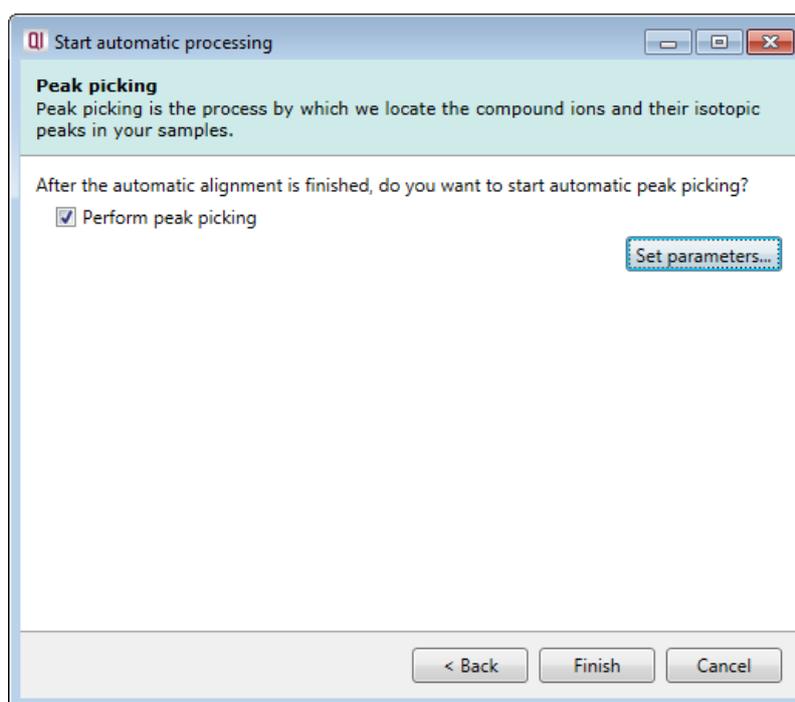


Figure 9: The **Peak picking** selection screen. The parameters are already optimised for this workflow but can be seen using **Set parameters**.

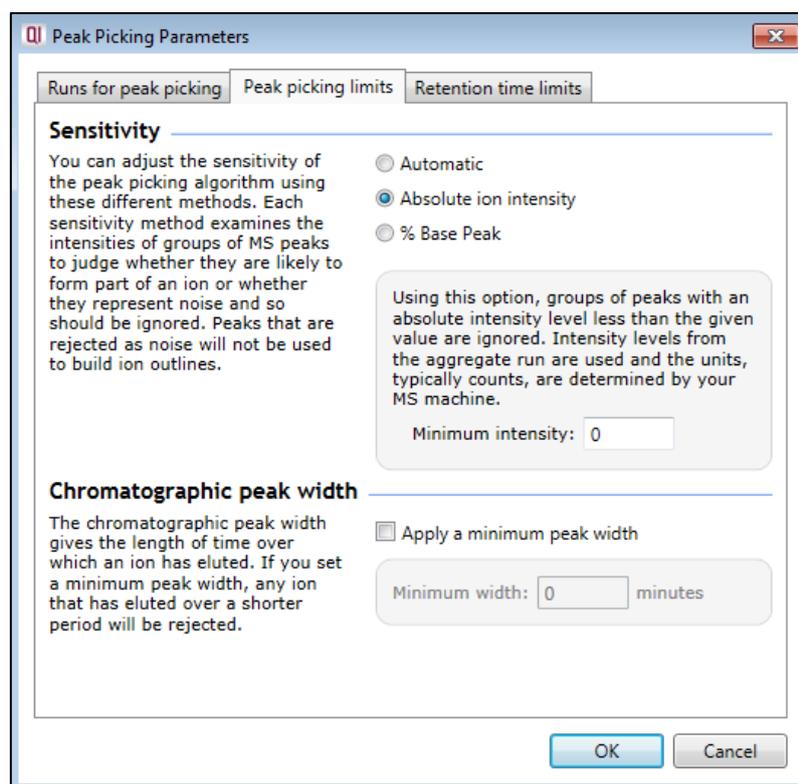


Figure 10: The ready-optimised parameters for peak picking; **Absolute ion intensity** is used for the sensitivity setting, with a minimum intensity of zero.

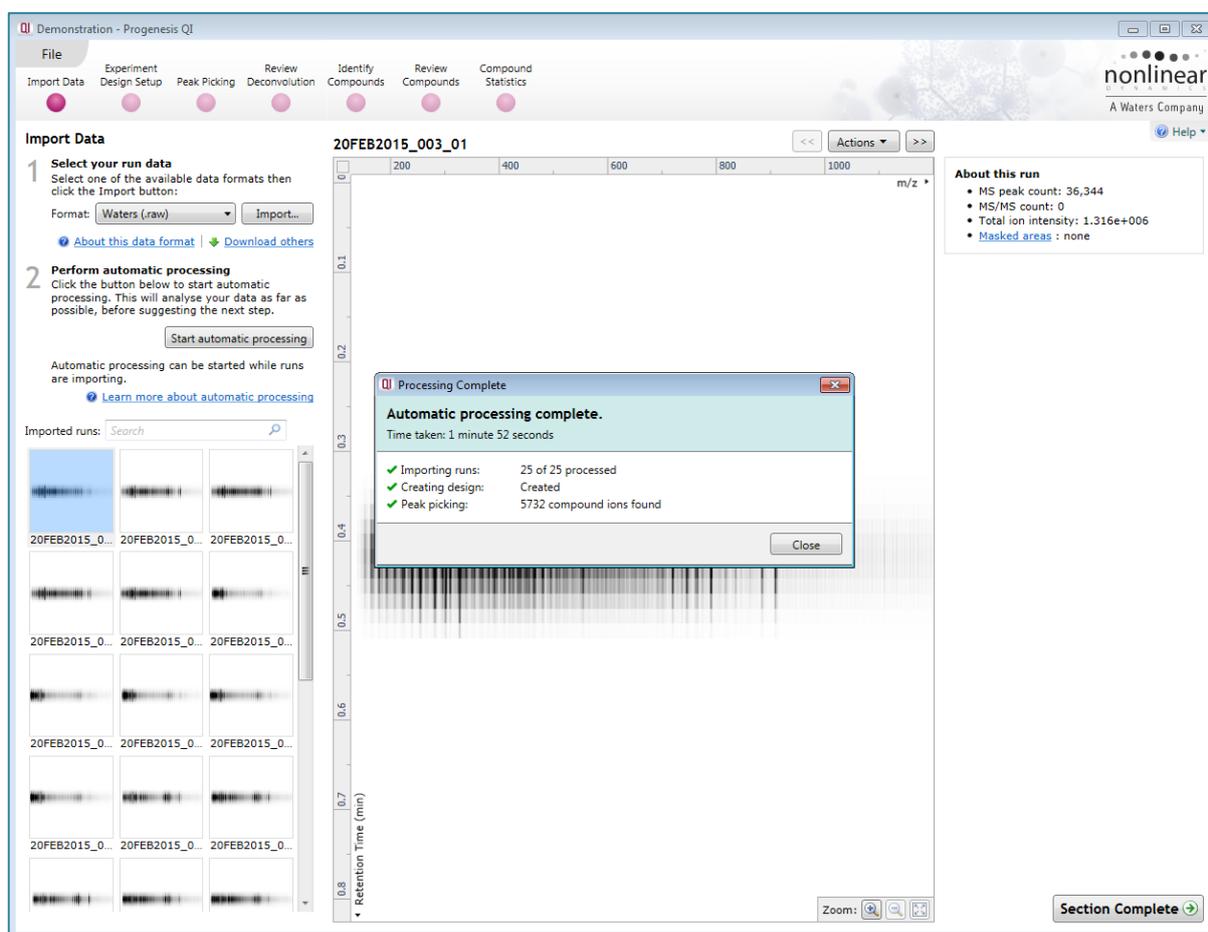


Figure 11: The results of automatic processing are displayed after completion. Closing this dialog allows you to review the results step-by-step.

Review Processing

Once the processing has completed and you have closed the dialog, you can step through the **Experiment Design Setup**, **Peak Picking** and **Review Deconvolution** screens by using the **Section Complete** button or the workflow buttons at the top of the screen.

All of these steps have been carried out to the extent necessary by the automatic processing ([Figures 12, 13 and 14](#)).

It is important to note that for Direct Sample Analysis data, there is no automatic deconvolution of adducts into compounds, as there is in the 'generic' workflow. However, you can still carry out manual deconvolution if you wish to assign different ions to the same compound. This process is explained in the main user guide, and it would require you to have selected all potentially relevant adduct types at the time of creating the experiment (only those types are made available at the **Review Deconvolution** step).

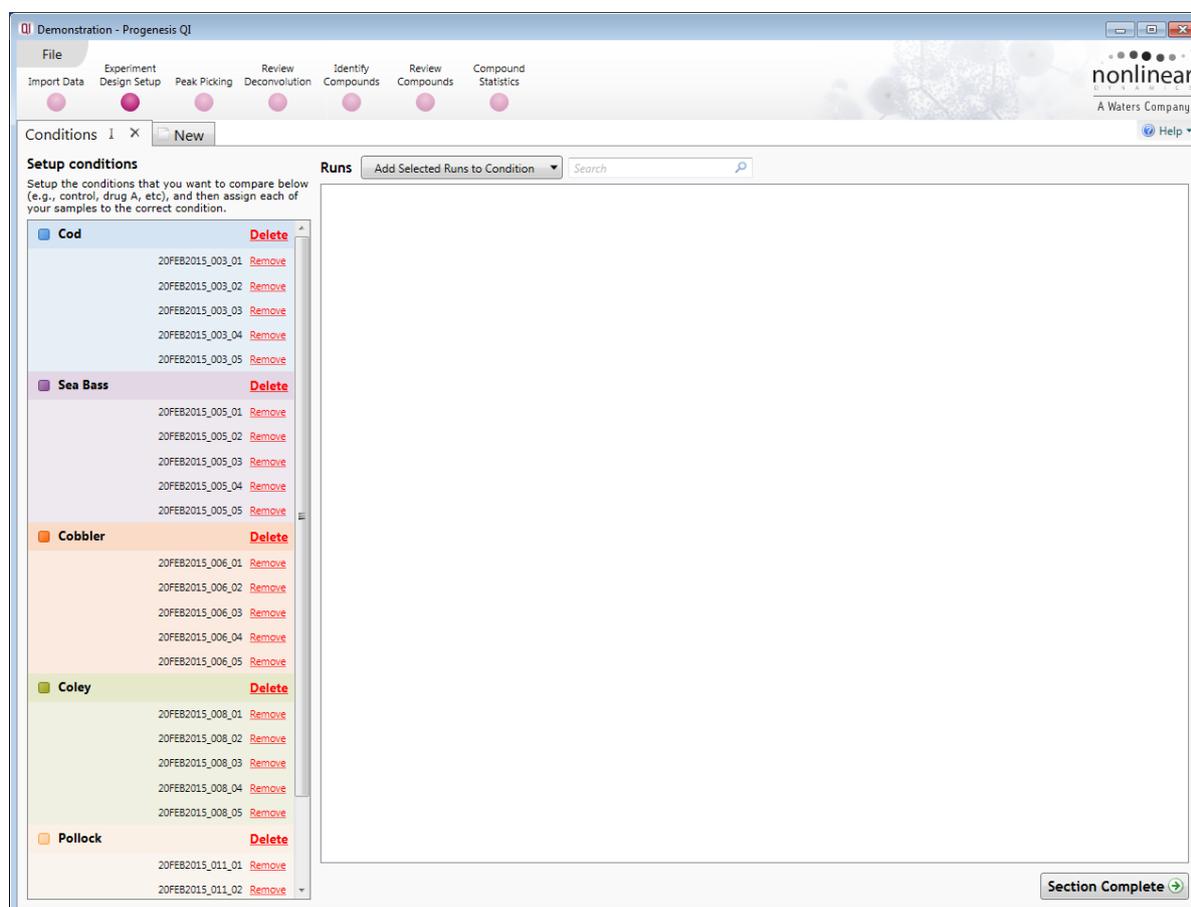
Review Processing – figures:

Figure 12: The groupings are already set up at **Experiment Design Setup**, although more may be added at this stage (and will be [later](#) in the guide for OPLS-DA analysis).

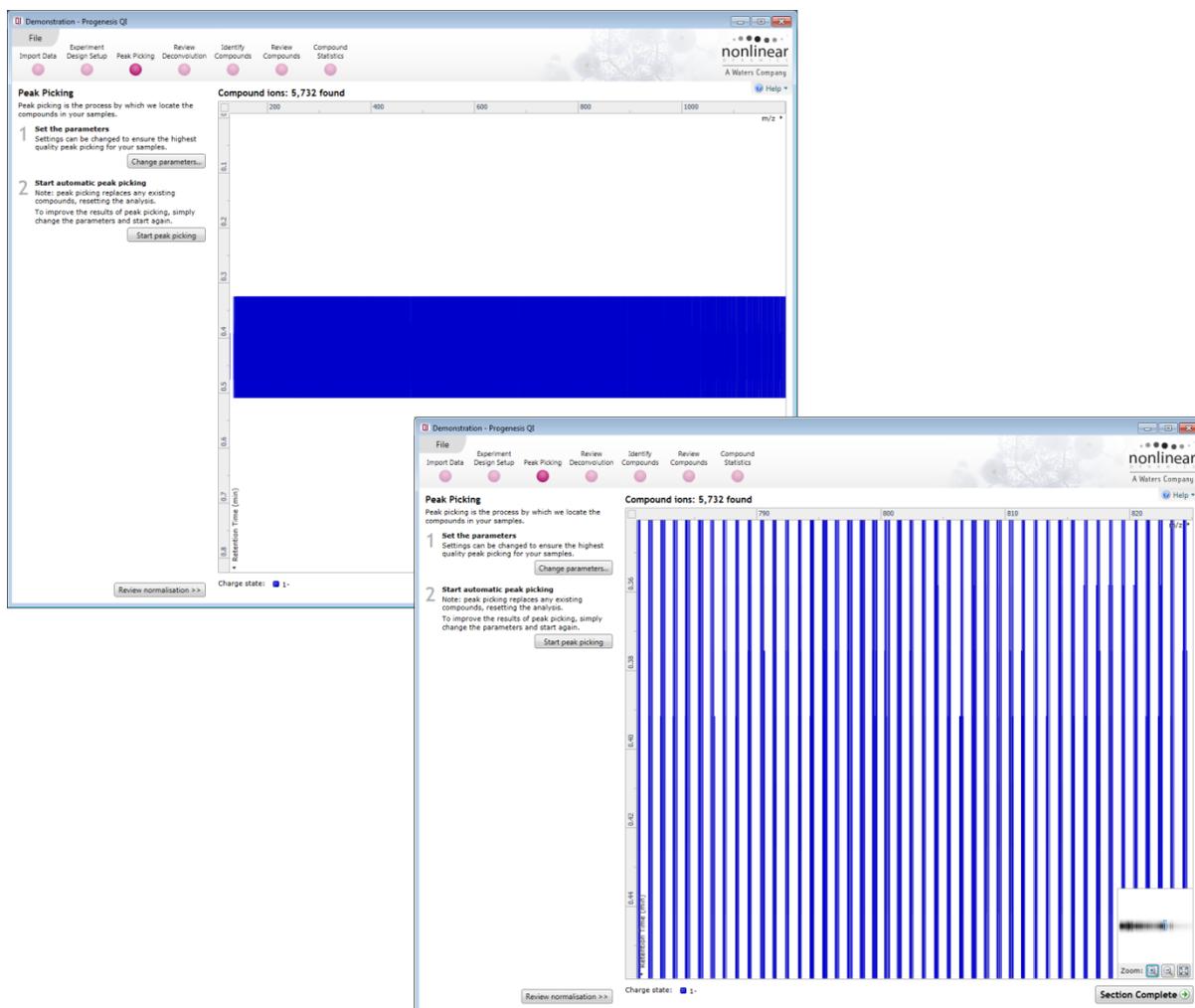


Figure 13: At **Peak Picking** the detected m/z profile is visible. The profile will potentially appear quite crowded (back panel), but on zooming in (front panel) separate ions can be distinguished.

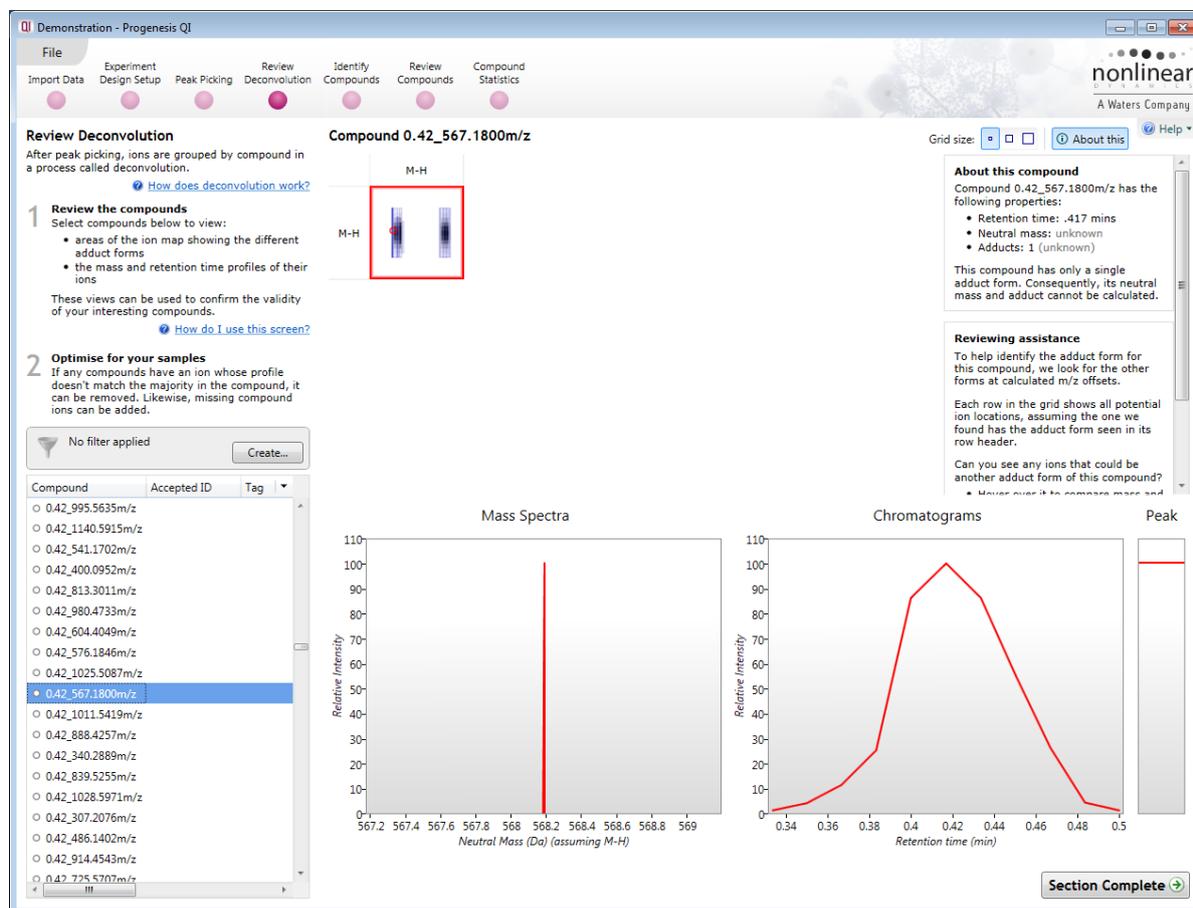


Figure 14: At **Review Deconvolution** potential adducts are shown for each compound. In this example data set, only [M-H] was defined on the creation of the experiment, so there are no other options. However, useful information is still available.

Initial Statistical Analysis

Progenesis QI has in-built statistical analysis tools, including multivariate visualisations such as Principal Component Analysis (PCA) and hierarchical clustering dendrograms, along with ANOVA analysis to detect significantly altered compounds. While these are described in detail [elsewhere](#), their use for the example data set is described below.

PCA separation of different fish species

1. Navigate to the **Compound Statistics** stage using the workflow button at the top of the interface, bypassing (for the moment) the **Identify Compounds** and **Review Compounds** stages.
2. PCA results will be calculated, and displayed ([Figure 15](#)).
3. Of particular note is the separation in the first two components of Pollock from all other species. We will return to this in the [next section](#).

Tagging of compounds that are significantly different between the groups for investigation

4. In the table on the left of the page, compounds are listed along with their ANOVA result for a comparison across all the groups ([Figure 16](#)).
5. To quickly select the compounds that are significantly different across the groups, and mark them for potential identification, right-click on this table and use **Quick Tags > ANOVA p-value...** to apply a tag label at any level you desire ([Figure 17](#)).
6. In this data set, most of the compounds are significantly altered between the samples, which are quite different in behaviour. Hence, we will instead focus on the discovery of compounds of interest by a second method, in the [next section](#).

Application suggestion: Speciation of an unknown sample by PCA

With an experiment containing authenticated samples, unknown samples can be added and compared to your standards to suggest a tentative grouping / classification.

If you do not wish to alter your current experiment with every such test, then first create an archive of the experiment from the main welcome screen, to save an unaltered copy you may return to at a later time.

Once this has been done:

1. Add the new run(s) at the **Import Data** stage.
2. Move forwards through the experiment, using **Section Complete** or the workflow buttons at the top of the page each time (*not* the automatic processing).
3. At **Experiment Design Setup**, create a new experimental condition group for the new run(s), and place them in it.
4. Continue onwards, accepting the existing peak picking when you pass the **Peak Picking** stage.

- When you reach the **Compound Statistics** screen, the new runs will appear as a new group on the PCA plot, and can be compared to the existing data grouping.

Note that the presence of the new runs will affect the plot for all runs, and so this is *not* any kind of rigorous classification mechanism. Also, your ANOVA values will be affected (and even lost, if you only add one new sample, as there are not enough replicates for ANOVA in all the groups any more).

This process is demonstrated using the demonstration data below ([Figure 18](#)). You may wish to try out the process yourself, using the file **20FEB2015_006_06.raw** in the **New Sample** folder in **Progenesis QI DSA Data.zip**. This is a Cobbler sample.

Initial Statistical Analysis – figures:

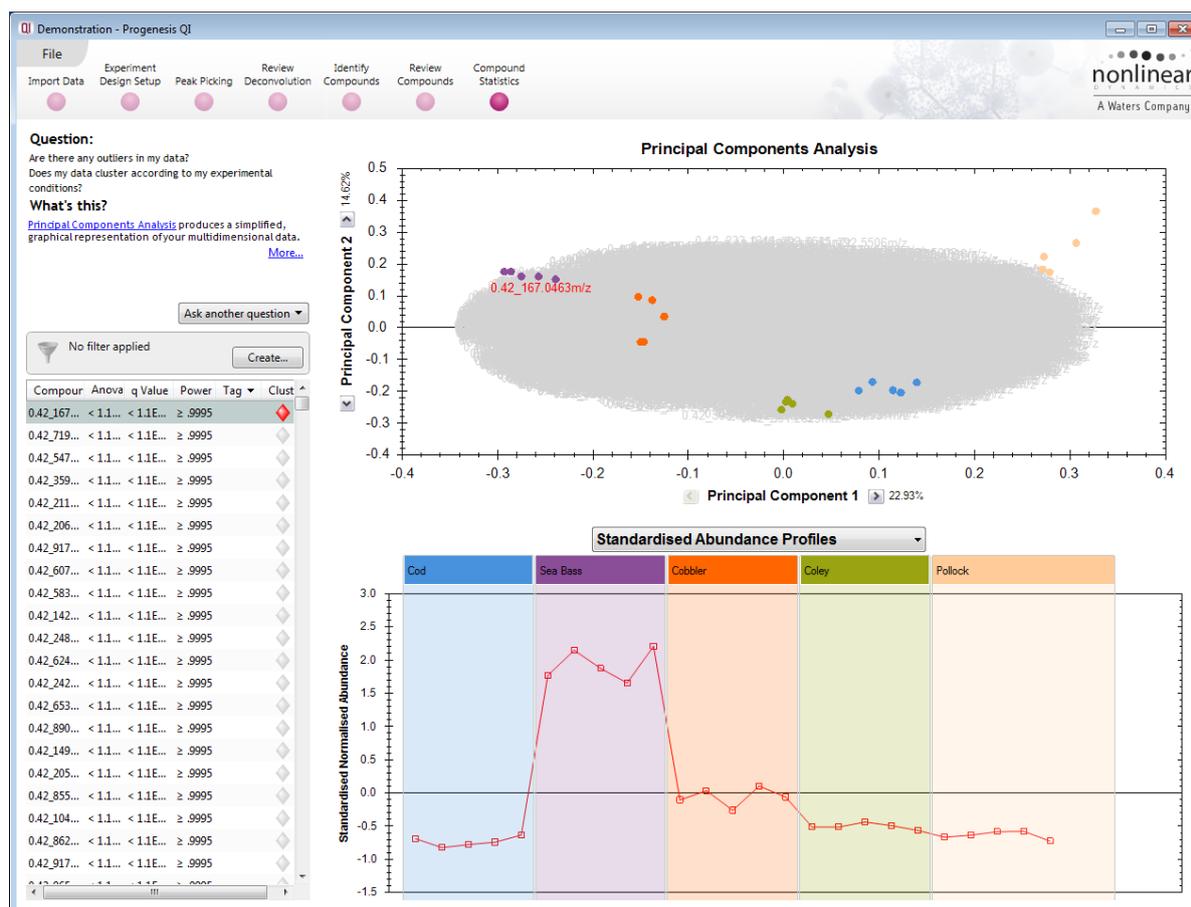
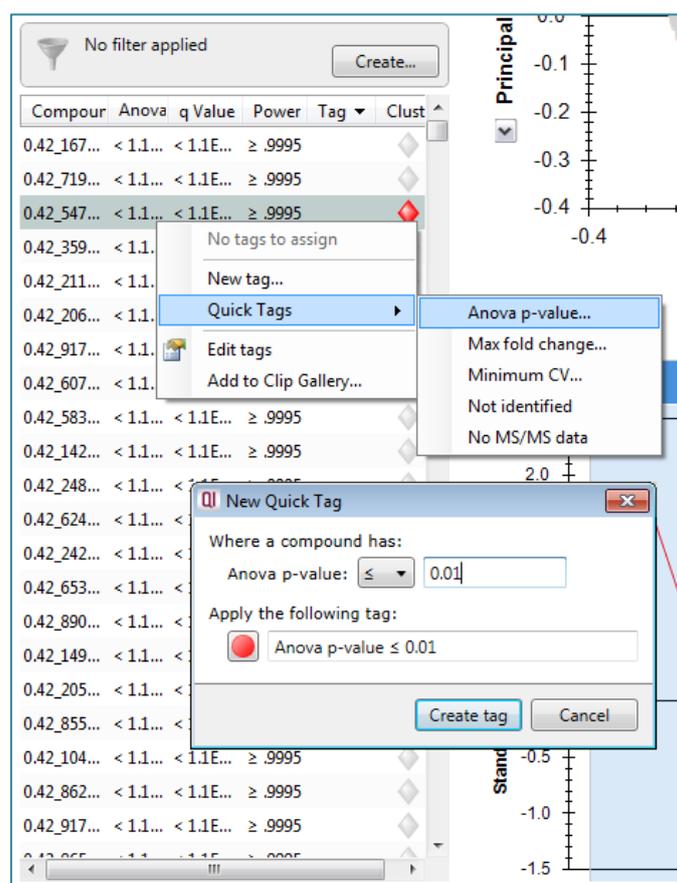


Figure 15: At **Compound Statistics** the separation of normalised compound abundance profiles by PCA can be seen.



| Compour | Anova | q Value | Power | Tag | Clust |
|-------------|----------|-----------|---------|-----|-------|
| 0.42_167... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_719... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_547... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_359... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_211... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_206... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_917... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_607... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_583... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_142... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_248... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_624... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_242... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_653... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_890... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_149... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_205... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_855... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_104... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_862... | < 1.1... | < 1.1E... | ≥ .9995 | | |
| 0.42_917... | < 1.1... | < 1.1E... | ≥ .9995 | | |

Figure 16: ANOVA values are presented in tabular form for each compound (note that for fold changes in the mean normalised compound abundance, see instead **Review Compounds**).



The screenshot shows a table of ANOVA values for various compounds. A context menu is open over the table, and a 'New Quick Tag' dialog box is displayed. The dialog box is titled 'New Quick Tag' and contains the following text:

Where a compound has:
Anova p-value: ≤ 0.01

Apply the following tag:
 Anova p-value ≤ 0.01

Buttons: Create tag, Cancel

Figure 17: Compounds whose mean normalised abundances differ significantly between the groups can easily be tagged for reference.

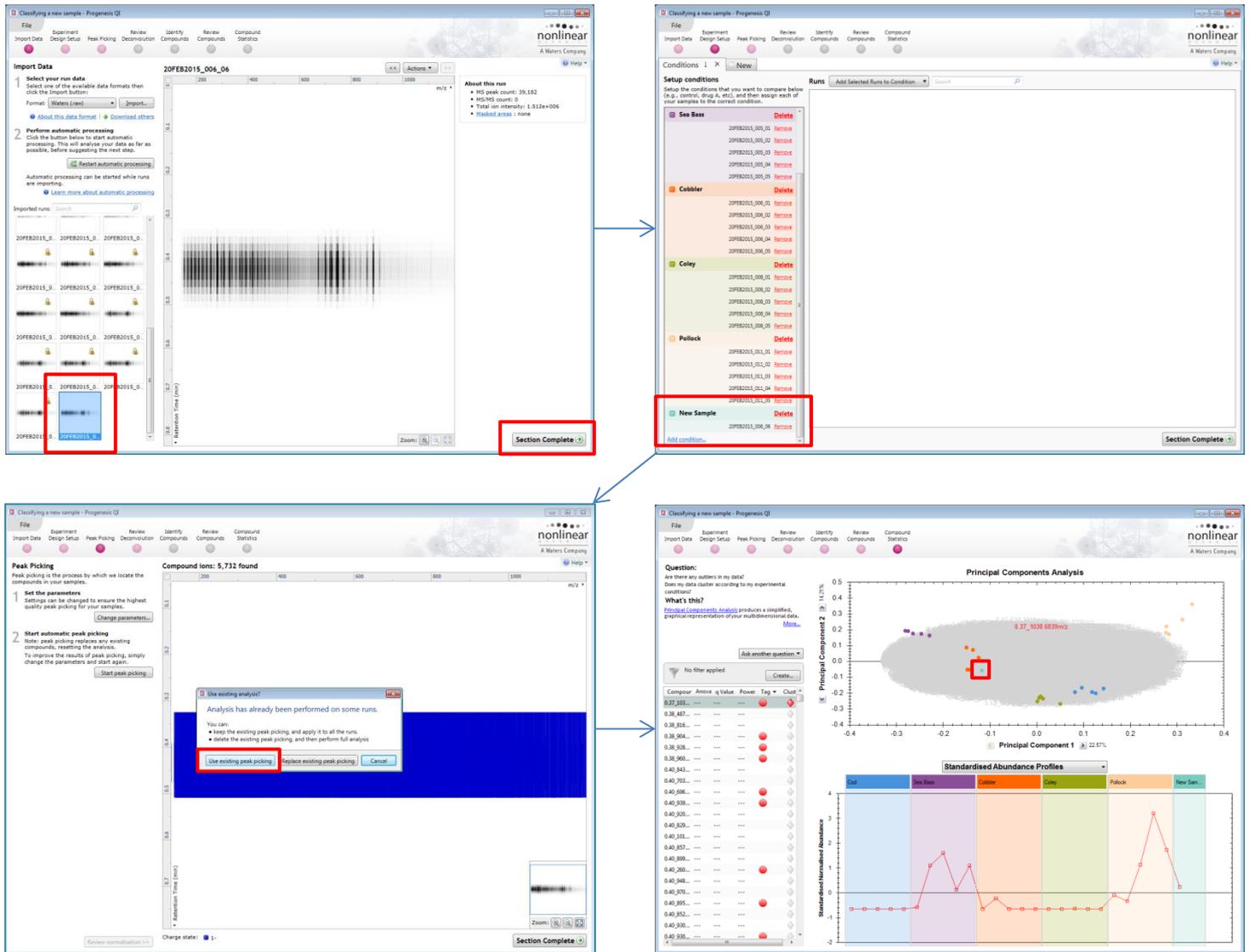


Figure 18: One may add new samples to an existing experiment, and using the existing detection, compare them consistently to the existing samples for (simplistic) classification purposes.

OPLS-DA Statistical Analysis Using EZinfo

Note: This requires EZinfo 3.0.3 statistical software, available for purchase with Progenesis QI.

Another way to find compounds of interest differing between groupings is the use of OPLS-DA (Orthogonal Projections to Latent Structures Discriminant Analysis). In this method, compound data are exported to EZinfo 3.0.3 statistical analysis software, which calculates the features that most effectively and robustly contribute to discrimination between two experimental groupings. These features can then be imported back into Progenesis QI as a tag set, and identified.

Please note that this section continues from the previous one *without* the optional addition of the [unknown](#) sample to the demonstration data. If you have added the new sample, you may return to an archived version of the experiment from before it was added to match this guide exactly, or simply continue with it present. You will see similar results whichever option you choose.

Generating an appropriate experiment design:

The first step is to generate a pair-wise comparison; that is, two groupings. In the case of the example data, our initial examination suggested that Pollock vs other samples might be an interesting comparison, given the visibly 'different' behaviour of that species on the PCA plot.

This requires formalising in an experiment design. To do this:

1. Return to the **Experiment Design Setup** stage.
2. Create a new **Between-subject Design** using the **New** tab. Select **Copy an existing design** ([Figure 19](#)) to create a copy of the existing fish groups.
3. Delete all the groups apart from the Pollock group, and then re-add all the other samples into a new condition ([Figure 20](#)).
4. Leaving this design selected (and so active), move back to the **Compound Statistics** stage.

Exporting data to EZinfo 3.0.3:

The next step is to export this comparison to EZinfo for external statistical analysis. Note that you should not have any tag filter applied at this stage (you want to export all the compounds for analysis).

5. At the **Compound Statistics** stage, select **File > Export to EZinfo...** ([Figure 21](#)).
6. At the pop-up prompt, save an EZinfo .usp file in an appropriate location. This is the record of the external statistical analysis; you may wish to also retain this file as well as the Progenesis experiment.
7. EZinfo will launch after compound export ([Figure 22](#)). The groupings, data and appropriate data scaling are already set up correctly in the workspace, so all that is needed is to perform the appropriate analysis.

Discriminant analysis in EZinfo 3.0.3:

We will apply an OPLS-DA analysis to the data to identify markers discriminating between Pollock and other fish species sampled in this study.

8. Open the **Model** tab and select **Change model**.
9. In the dialog, select **Analyse group differences by** “Condition” and select “OPLS-DA” ([Figure 23](#)). Whether you create the analysis as a new model or not is not important for the purpose of this demonstration (in this case, the box is unticked, so replacing the initial model).
10. Now, select **Plots and Spreadsheets**, and the **S-Plot** ([Figure 24](#)).
11. In principle, the compounds at the extremes of an S-Plot represent species that contribute highly to the discriminant separation model with high reliability. These are therefore your potential biomarkers. Select some by clicking and dragging (this is only a demonstration, and the exact species selected do not greatly matter). If selecting multiple regions, holding Ctrl is necessary to avoid only selecting the latest group.

An example is provided ([Figure 25](#)).

12. Select **Transfer Loadings data** on the side-bar ([Figure 25](#)).
13. Close the EZinfo window, saving if desired.

Importing the compounds of interest into Progenesis QI:

14. Your selected species will be ready for import in the **Collecting data from EZinfo** window. Assign a tag to them as desired, creating it if necessary ([Figure 26](#)). Right-clicking the compound row, and then selecting the desired tag will then apply it to the compounds ([Figure 26](#)).
15. Click **Import Tags**.
16. You will be prompted to close the connection to EZinfo on import – confirm this.
17. Your tags will be applied, and you have marked the species contributing most notably to OPLS-DA discrimination between Pollock and other fish species.
18. You can apply a tag filter to only show these potential biomarkers ([Figure 27](#)). These will form a good sub-selection for attempted compound identification in the [next section](#).

OPLS-DA using EZinfo – figures:

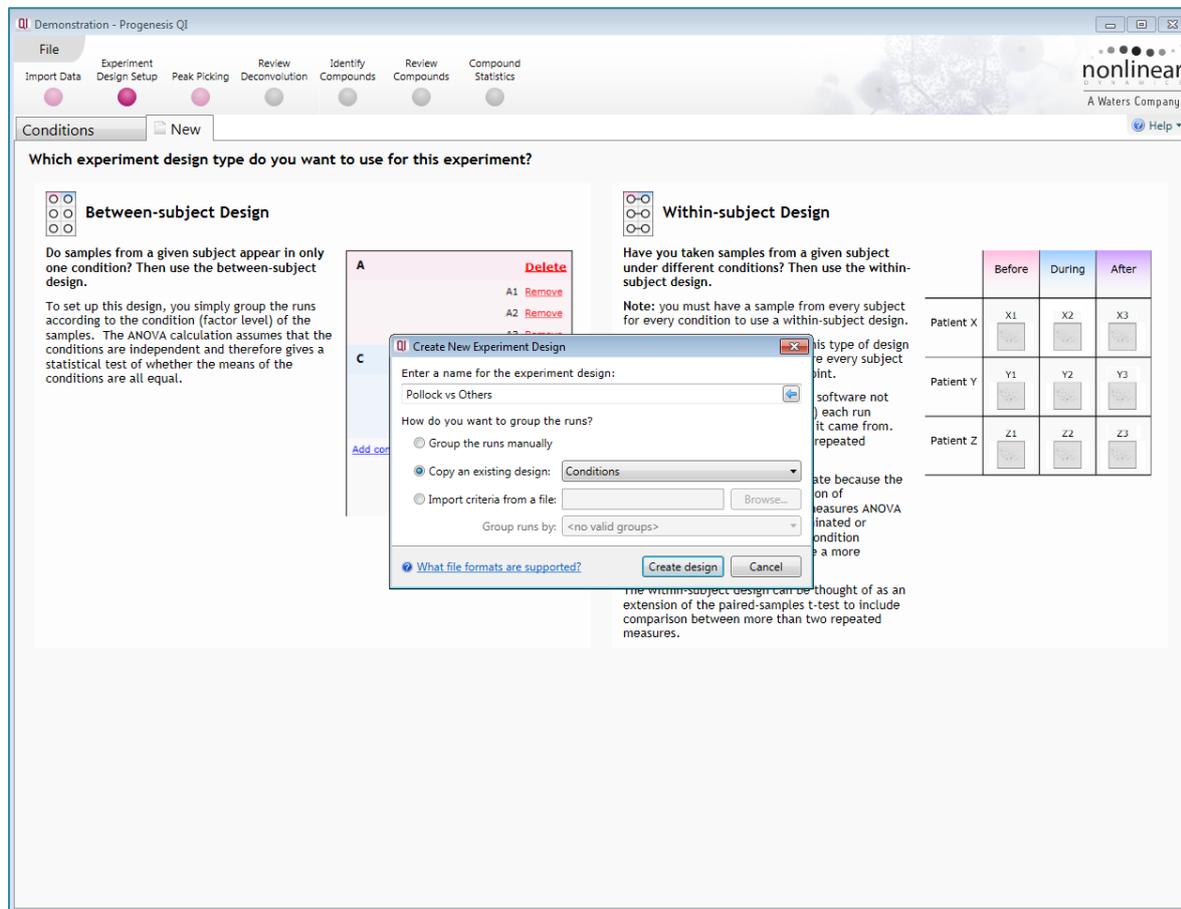


Figure 19: Create a new experiment design, copying the existing one that was generated by the template file for convenience.

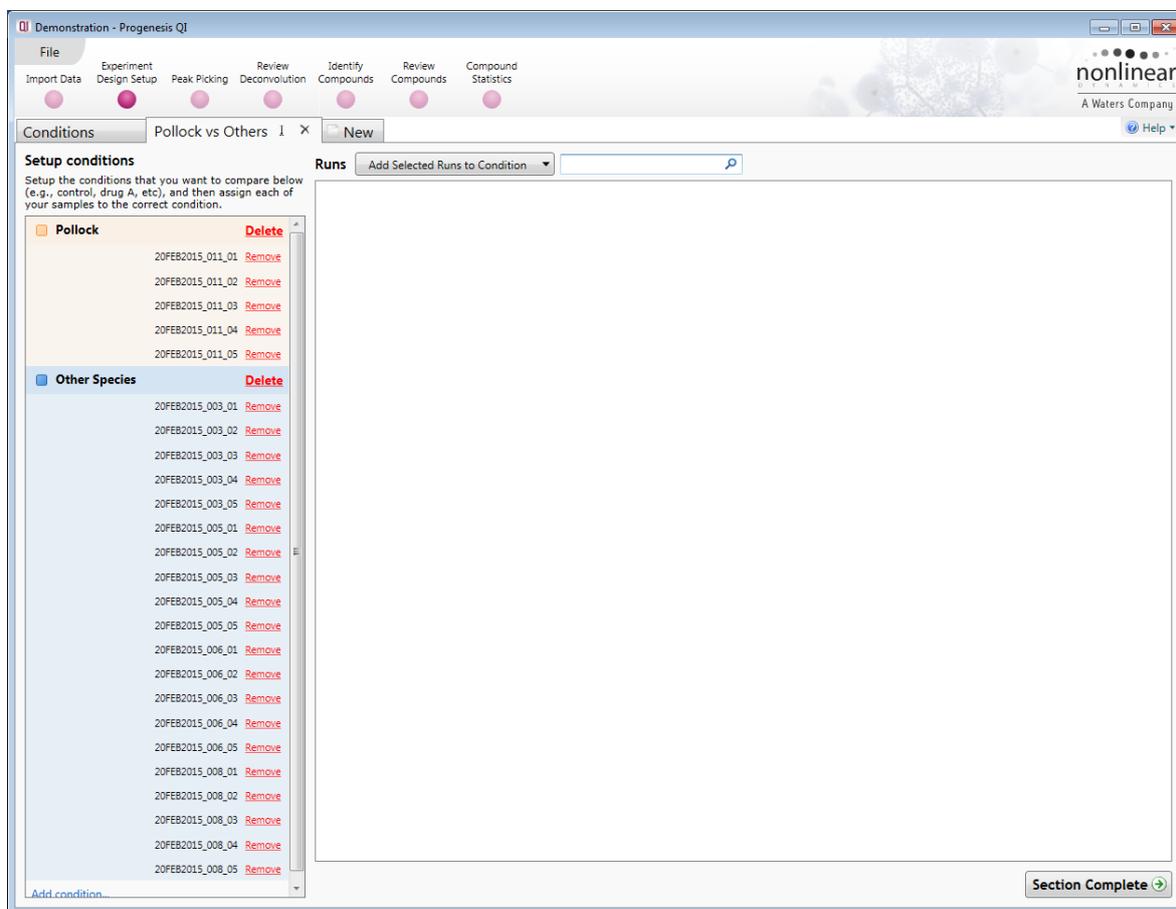


Figure 20: Remove all the groups except Pollock using **Delete** on each group, and then re-add all the 'freed' samples into a single comparison group.

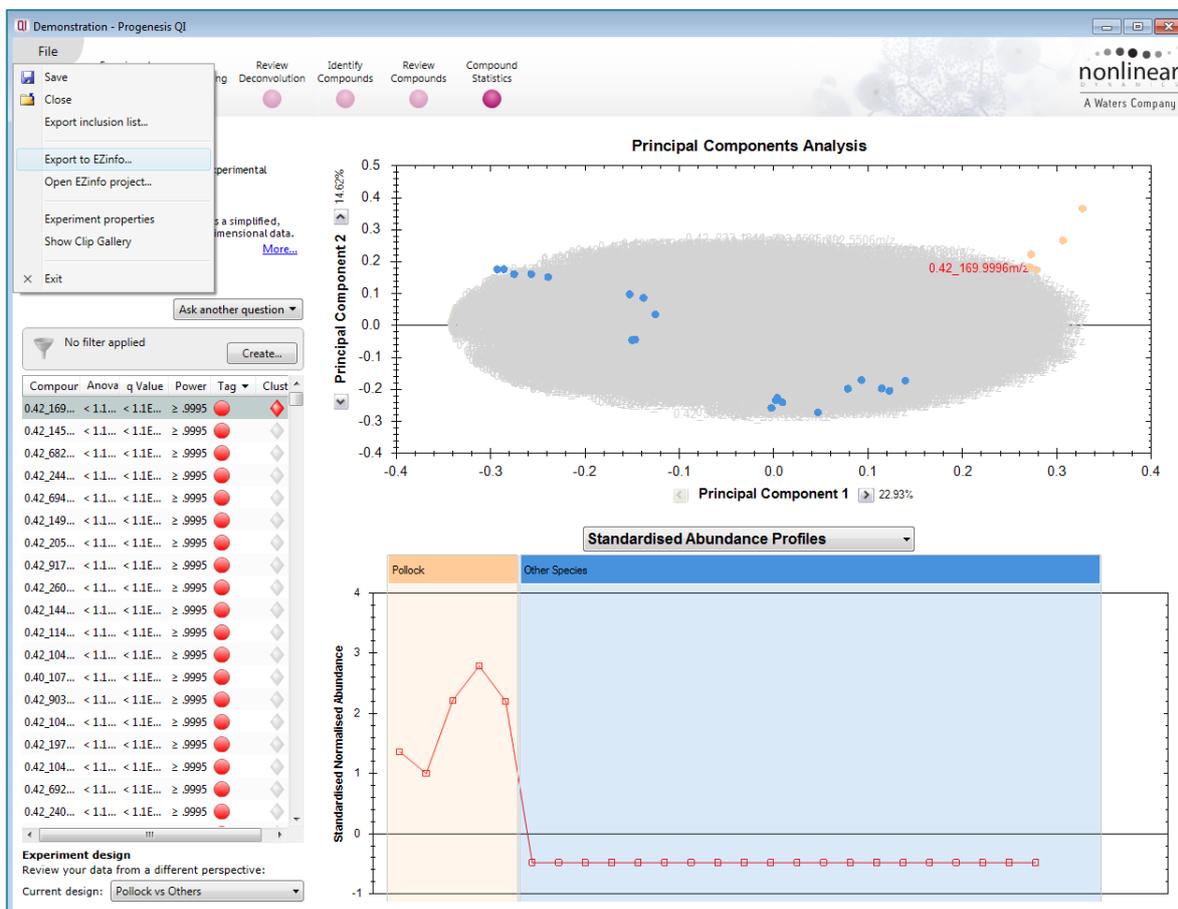


Figure 21: Return to **Compound Statistics**, and export the data to EZinfo. Note the PCA appears identical (the same samples are being plotted) with the exception that all other samples form one 'super-group'; only the group colouring changes on the plot.

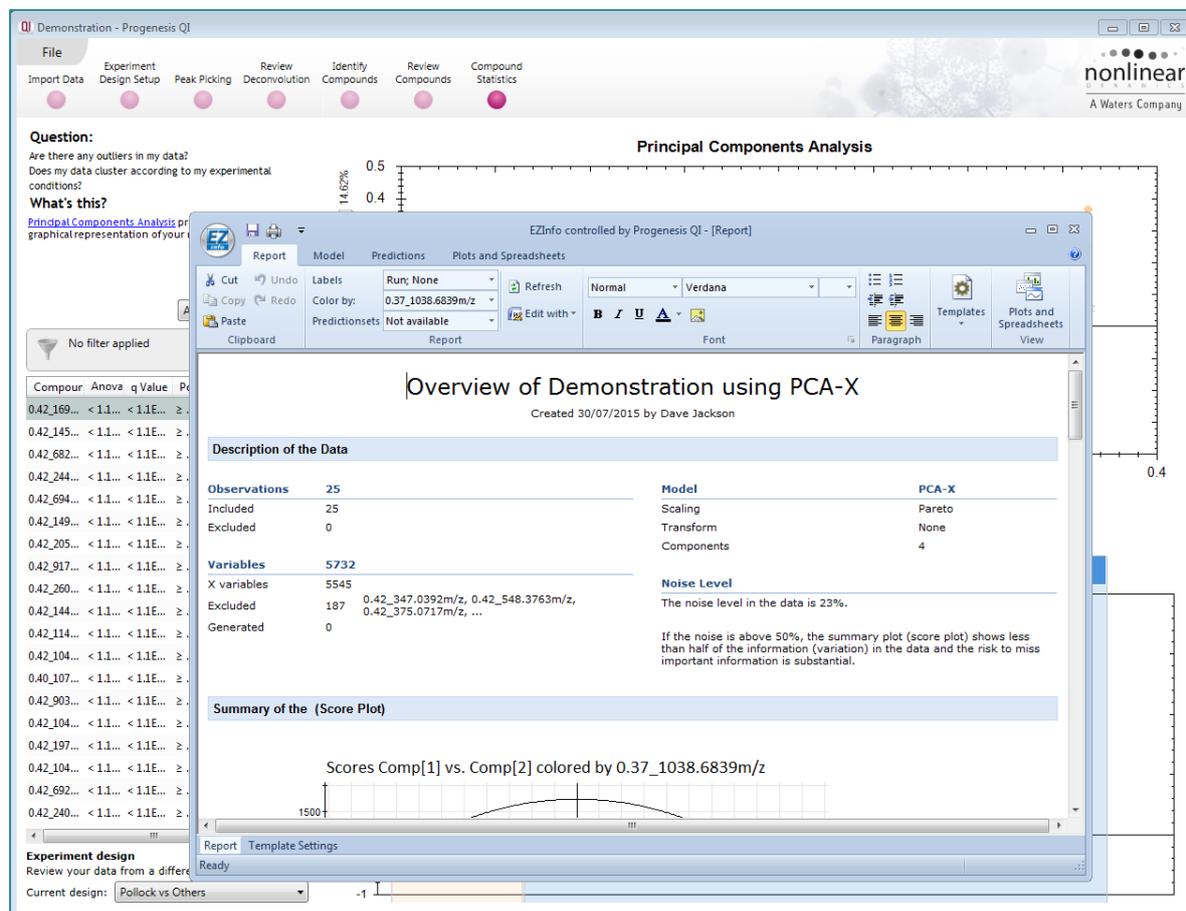


Figure 22: EZInfo will launch (after saving a usp file as prompted), with the data being ready for OPLS-DA analysis. Carrying out the analysis itself will be the next step.

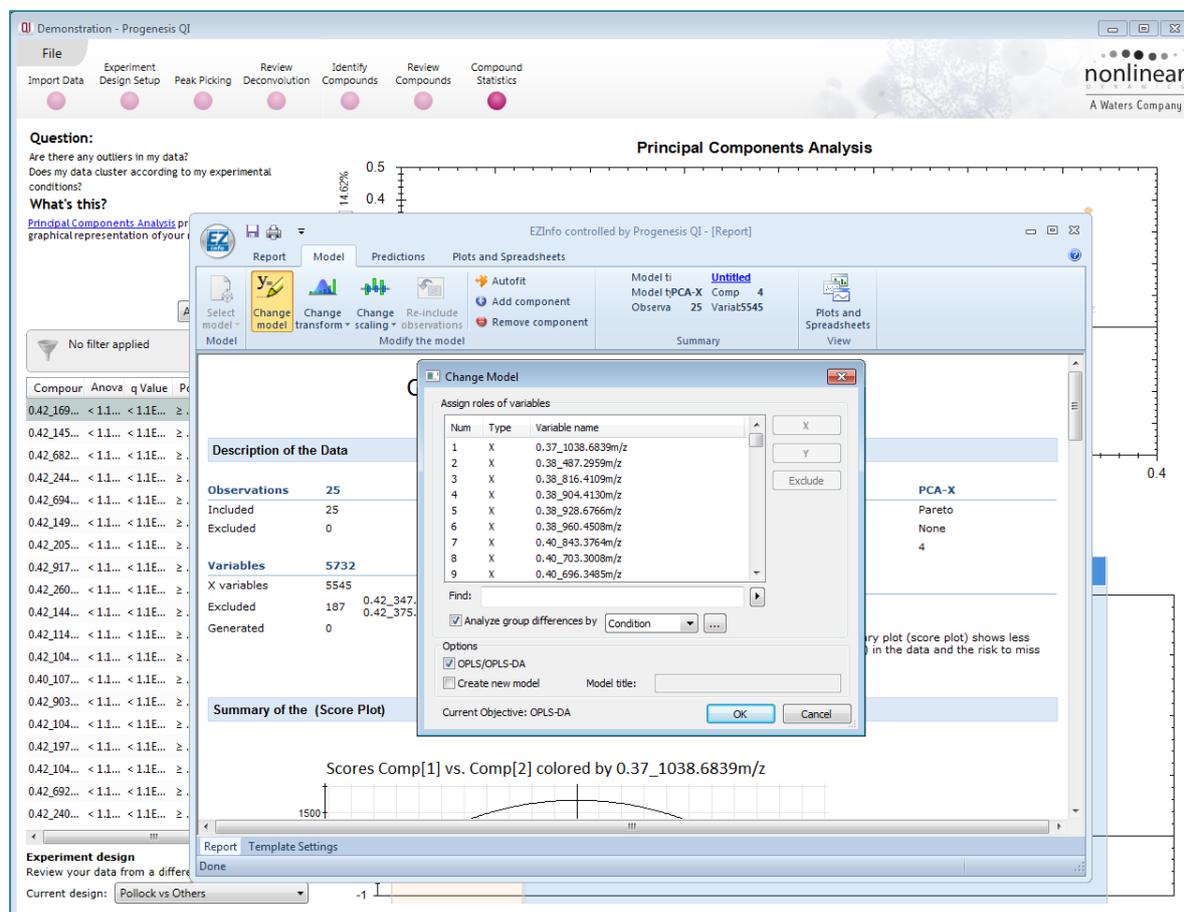


Figure 23: From the **Model** tab, select **Change model**. In the **Change Model** box, select to analyze group differences by condition, and select OPLS-DA.

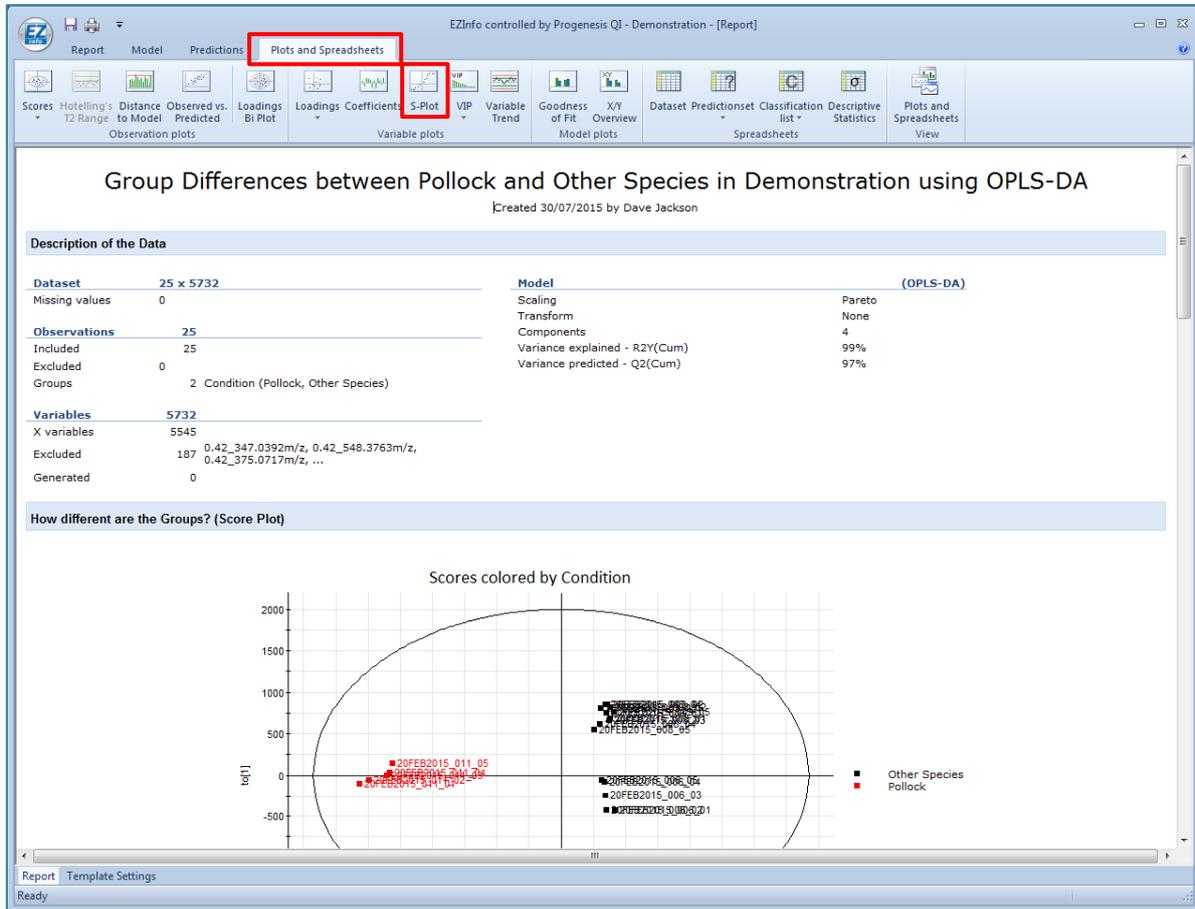


Figure 24: OPLS-DA analysis will be performed. The next step will be to select potential biomarkers contributing strongly to the discriminant model. Select **Plots and Spreadsheets** and **S-Plot** as shown.

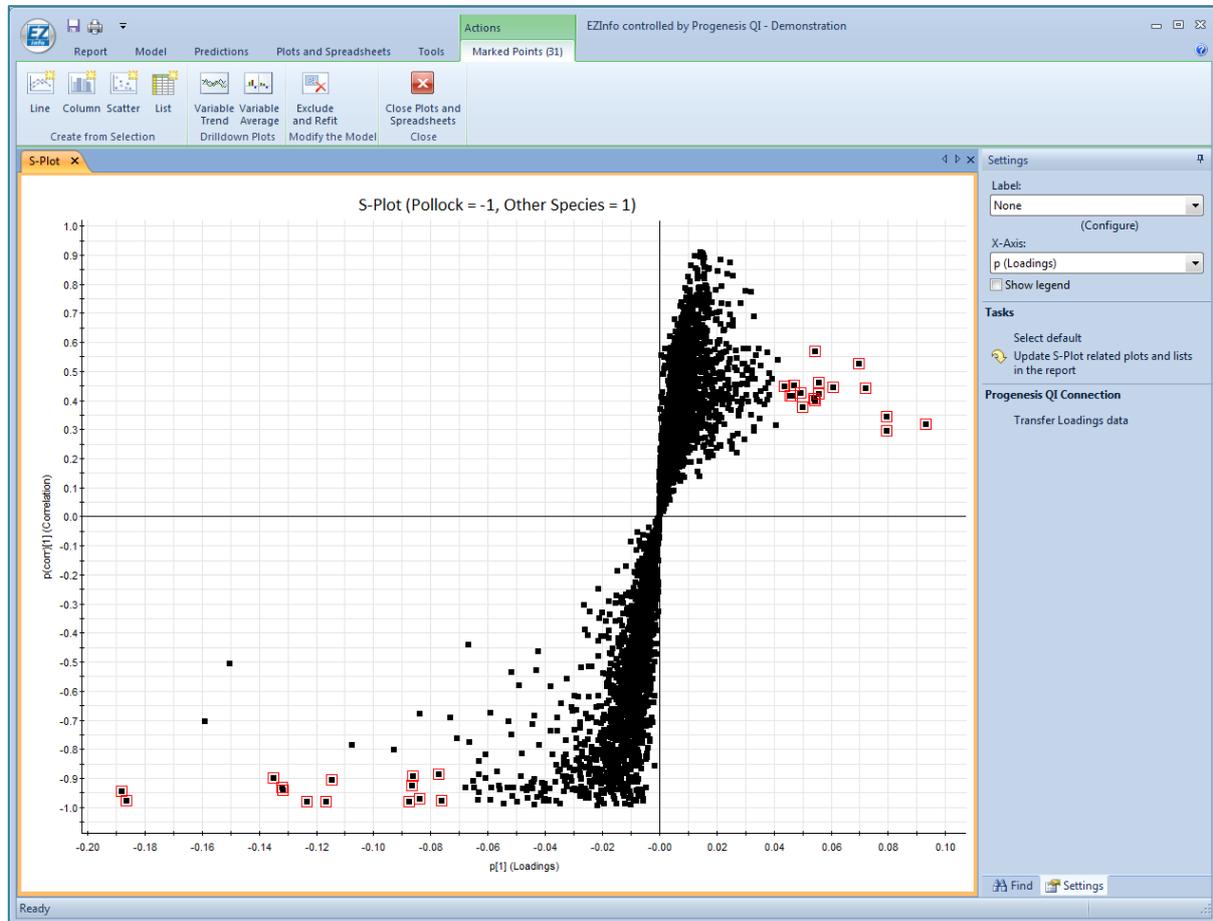


Figure 25: Once **S-Plot** is selected from the **Plots and Spreadsheets** tab, the plot appears and you can select potential biomarkers from the extremes of the 'S'. To select multiple groups, hold **Ctrl** while clicking and dragging. Once you have a satisfactory set, select **Transfer Loadings data** on the right-hand side-bar.

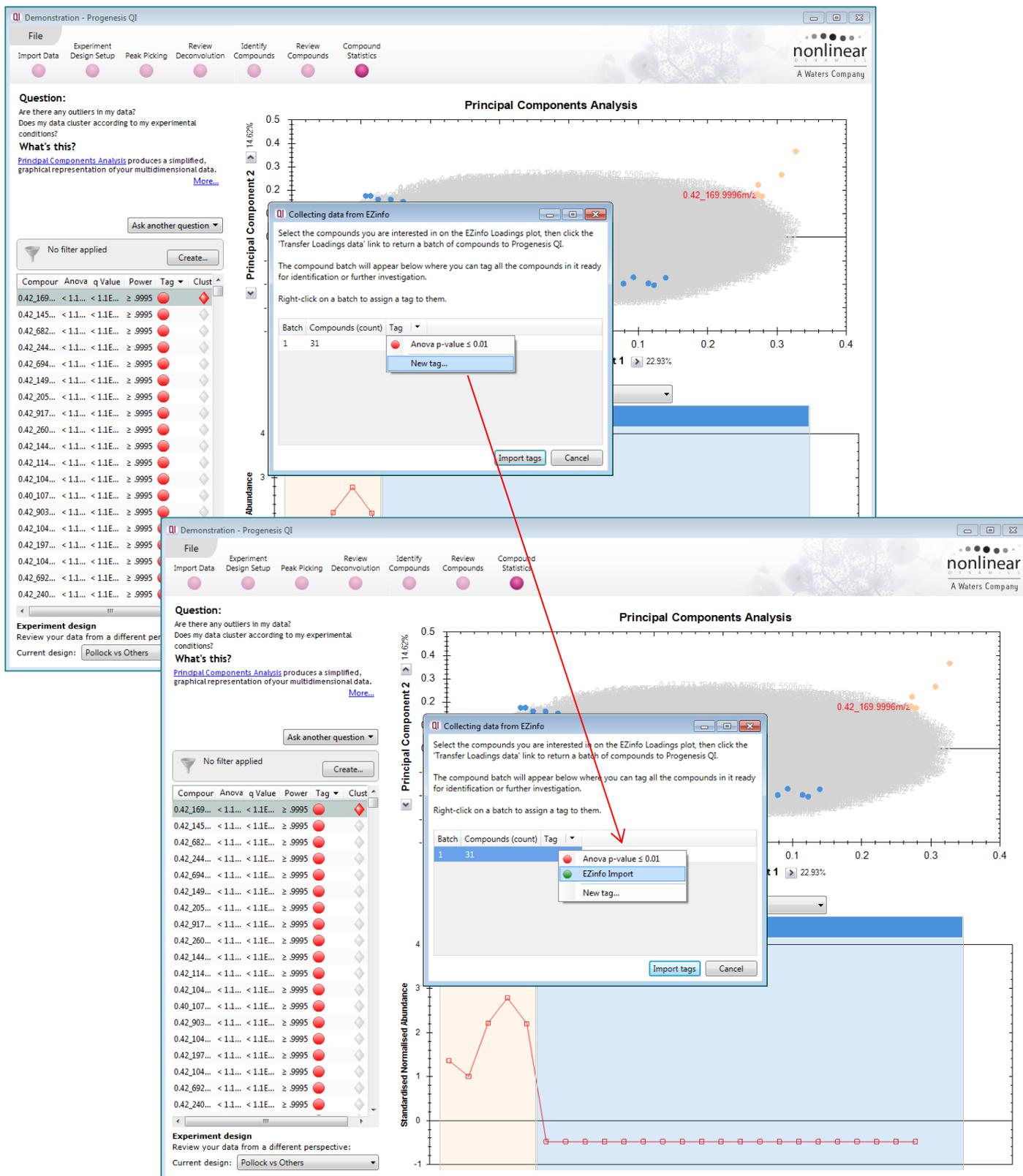


Figure 26: On closing EZinfo, you are returned to Progenesis QI. Using the black arrow drop-down, create a tag for the import (if you have not created a suitable one already). Then right-click on the compound batch line, and apply that tag. The compounds can now be imported under that tag label.

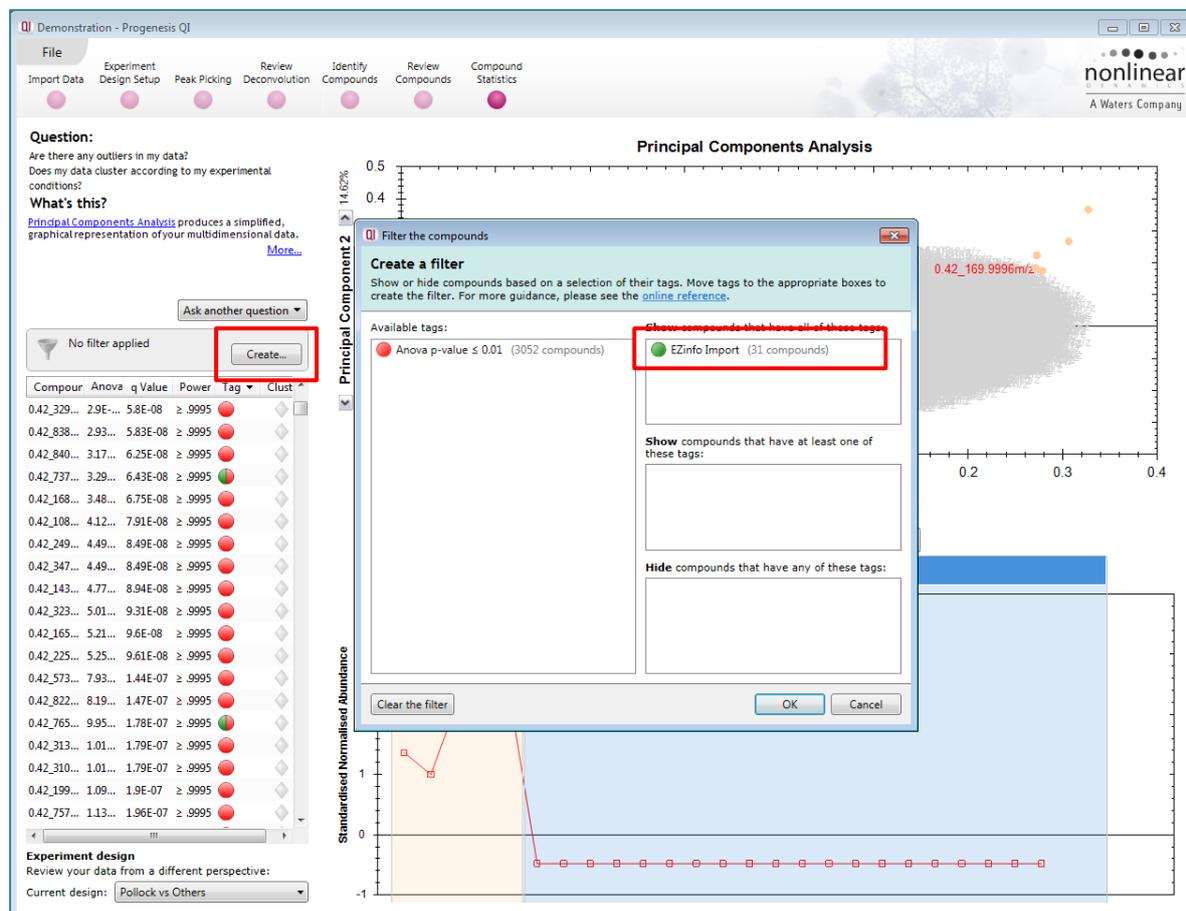


Figure 27: Use the filtering function to show only the imported compounds.

Compound Identification

Progenesis QI allows a number of search methods for biomarker identification. These include native support for ChemSpider, LipidBlast, Metlin and NIST. In addition, the Progenesis MetaScope search engine allows searching of any SDF or CSV database, e.g. those provided by HMDB, DrugBank and LipidMaps.

For REIMS Direct Sample Analysis data, such as those in the example set, one would expect to predominantly see lipid species, so searching LipidBlast and the LipidMaps SDF file through Progenesis MetaScope are sensible starting points.

However, both methods carry the caveat that for TOF-MS data, the lack of fragment information can make determining the correct identification amongst several putative 'hits' challenging.

ChemSpider can also be used, but a great many putative identifications may be returned; the noted caveat is likely to be even more of an issue in this case. For more information on ChemSpider, see the online FAQs as detailed [previously](#); only the LipidBlast and MetaScope methods are detailed in this guide.

In all cases, to address the challenge of the lack of MS/MS data, DDA enhancement may help, and the process for achieving this is also described below.

Identification using LipidBlast:

Note that if this is the first time you have used LipidBlast, you will need to install the necessary databases. This is easy (and indeed automatic if you have internet access), as described in [online FAQ](#) material.

1. Move to the **Identify Compounds** stage, with the tag filter still active from the EZinfo import in the previous section (that is, you are only seeing the compounds that you highlighted on import from EZinfo).
2. Select **LipidBlast** from the drop-down menu ([Figure 28](#)).
3. You can alter the tolerances if desired, but in this example the defaults will be used (5ppm for both precursor and fragment tolerances).
4. Click **Search for identifications**.
5. Putative identifications will be imported where available ([Figure 29](#)).

Identification using the LipidMaps SDF in Progenesis MetaScope:

For this method, you will require the structure SDF file available at [LipidMaps](#). The file required is LMSDFDownload6Apr15FinalAll.sdf (or current version dependent on date), which is present in the downloadable structure archive (e.g. LMSDFDownloadApr15.zip).

6. Select **Progenesis MetaScope** from the same drop-down menu as step 2.
7. Under **Choose search parameters**, select **Edit > Create New**.

8. Create a search parameter set using the LipidMaps sdf file. Suitable parameters are shown in [Figure 30](#); the set should be named, and the sdf selected in the **Compound database** field, but no other changes are necessary.
9. Save the set using the **Create search parameters** button.
10. From the main search menu, ensure this search method is selected, and click **Search for identifications**.
11. As with the previous LipidBlast search, results will be imported where hits occur ([Figure 31](#)).
12. Each method is assigned a letter, so that you can differentiate between results from the LipidBlast search (“A” in this case) and LipidMaps MetaScope search (“B” in this case) ([Figure 32](#)).

For more information on other identification methods, please consult the main user guide and the online FAQ material, as detailed [previously](#).

Application suggestion: DDA enhancement

For the REIMS data shown here, there is no fragmentation information; as noted, this makes specific identification more challenging. If you wish, you can improve your identification confidence and specificity by adding in information from separate DDA (Data-Dependent Acquisition) runs. Progenesis QI can then carry out a more detailed identification process utilising fragment information.

To help with this, you can generate targeted inclusion lists for those runs.

From the **File** menu, then select **Export inclusion list...** and a pop-up dialog will allow you to select the Masslynx format and export the list ([Figure 33](#)). This will then allow you to acquire targeted DDA data for all the species currently present in your tag filter.

To use the information gained from DDA runs in your identification process, please consult the main user guide, [as described previously](#).

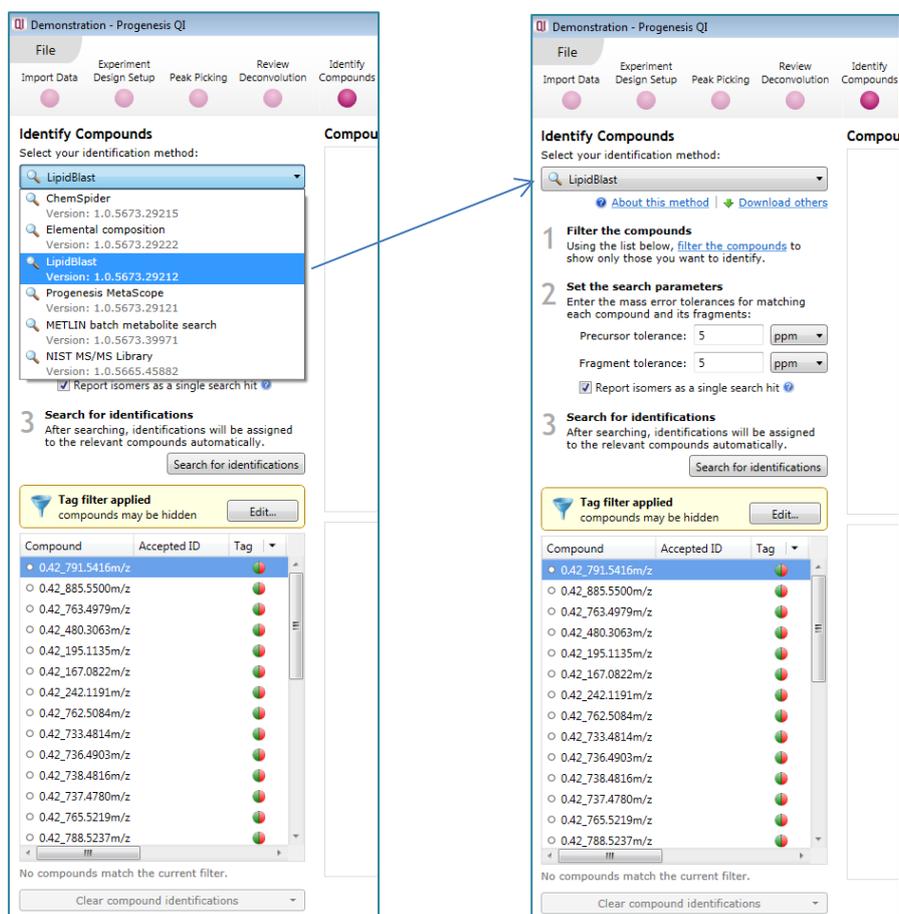
Compound identification – figures:

Figure 28: Selection of the LipidBlast search method at **Identify Compounds**, and the parameter selection dialog.

Identify Compounds
Select your identification method:
LipidBlast
[About this method](#) | [Download others](#)

- Filter the compounds**
Using the list below, [filter the compounds](#) to show only those you want to identify.
- Set the search parameters**
Enter the mass error tolerances for matching each compound and its fragments:
Precursor tolerance: 5 ppm
Fragment tolerance: 5 ppm
 Report isomers as a single search hit
- Search for identifications**
After searching, identifications will be assigned to the relevant compounds automatically.
[Search for identifications](#)

Tag filter applied
compounds may be hidden [Edit...](#)

| Compound | Accepted ID | Tag |
|------------------|-------------|-----|
| 0.42_791.5416m/z | | |
| 0.42_885.5500m/z | | |
| 0.42_763.4979m/z | | |
| 0.42_480.3063m/z | | |
| 0.42_195.1135m/z | | |
| 0.42_167.0822m/z | | |
| 0.42_242.1191m/z | | |
| 0.42_762.5084m/z | | |
| 0.42_733.4814m/z | | |
| 0.42_736.4903m/z | | |
| 0.42_738.4816m/z | | |
| 0.42_737.4780m/z | | |
| 0.42_765.5219m/z | | |
| 0.42_788.5237m/z | | |

8 of 31 filtered compounds have been identified.
[Clear compound identifications](#)

Compound 0.42_733.4814m/z

This compound has no fragmentation data

Possible identifications: 6

| Compound ID | Description | Adducts | Formula | Retention time | Score | Fragme |
|----------------|----------------|---------|--|----------------|-------|--------|
| GPA(17:2/22:4) | GPA(17:2/22:4) | M-H | C ₄₂ H ₇₁ O ₈ P | | 32.4 | 0 |
| GPA(17:0/22:6) | GPA(17:0/22:6) | M-H | C ₄₂ H ₇₁ O ₈ P | | 32.4 | 0 |
| GPA(22:6/17:0) | GPA(22:6/17:0) | M-H | C ₄₂ H ₇₁ O ₈ P | | 32.4 | 0 |
| GPA(17:1/22:5) | GPA(17:1/22:5) | M-H | C ₄₂ H ₇₁ O ₈ P | | 32.4 | 0 |
| GPA(22:5/17:1) | GPA(22:5/17:1) | M-H | C ₄₂ H ₇₁ O ₈ P | | 32.4 | 0 |
| GPA(22:4/17:2) | GPA(22:4/17:2) | M-H | C ₄₂ H ₇₁ O ₈ P | | 32.4 | 0 |

No structure data

Section Complete

Figure 29: Example results obtained for compound identification from LipidBlast.

Create New Search Parameters

MetaScope search parameters
Define a set of MetaScope parameters that can be saved for later reuse. Learn more in the [online reference](#).

Name: LipidMaps Search

Compound database: C:\Progenesis Files\LMSDFDownload6Apr15Final

Data format: Auto-detect

Search parameters

Precursor tolerance: 5 ppm

Retention time within: 0.1 minutes

CCS within: 2.5 %

Additional compound properties source

Read additional compound properties from this file

<no database selected>

Fragment search method

Do not use fragmentation data

Perform theoretical fragmentation

Fragment tolerance: 5 ppm

Perform fragment database search

<no database selected>

Fragment tolerance: 5 ppm

Figures 30 (above) and 31 (below): The MetaScope set-up dialog with the LipidMaps sdf file selected ready for searching, and an example LipidMaps MetaScope search result.

Demonstration - Progenesis QI

File Experiment Design Setup Peak Picking Review Deconvolution Identify Compounds Review Compounds Compound Statistics

Identify Compounds

Select your identification method:
Progenesis MetaScope

1 Filter the compounds
Using the list below, filter the compounds to show only those you want to identify.

2 Choose search parameters
Select your MetaScope search parameters or create a new parameter set:
LipidMaps Search

3 Search for identifications
Identifications will be assigned to the relevant compounds automatically.

Tag filter applied
compounds may be hidden

| Compound | Accepted ID | Tag |
|------------------|-------------|-----|
| 0.42_736.4903m/z | | |
| 0.42_738.4816m/z | | |
| 0.42_737.4780m/z | | |
| 0.42_765.5219m/z | | |
| 0.42_788.5237m/z | | |
| 0.42_792.5506m/z | | |
| 0.42_207.1497m/z | | |
| 0.42_391.2247m/z | | |
| 0.42_283.2621m/z | | |
| 0.42_253.2172m/z | | |
| 0.42_165.1032m/z | | |
| 0.42_255.2331m/z | | |
| 0.42_281.2487m/z | | |
| 0.42_279.2338m/z | | |
| 0.42_151.0876m/z | | |
| 0.42_155.0826m/z | | |

14 of 31 filtered compounds have been identified.

Compound 0.42_391.2247m/z

This compound has no fragmentation data

Possible identifications: 1

| Compound ID | Description | Adducts | Formula | Retention time | Score | Frag |
|-------------|-------------|---------|--|----------------|--------|------|
| 123060599 | CPA(16:0) | M-H | C ₁₆ H ₃₁ O ₆ P | | B 35.5 | B |

CCCCCCCCCCCCCCCC(=O)OP(=O)(O)O

Section Complete

Possible identifications: 12

| Compound ID | Description | Adducts | Formula | Retention time | Score | Fragmentation score | Mass error (ppm) | Retention time error (mins) | Isotope similarity | Link | Search Configuration |
|----------------|-----------------------------|---------|--|----------------|--------|---------------------|------------------|-----------------------------|--------------------|-------------|--|
| 123067054 | PA(17.0/22.6/42.7/10Z,13Z) | M-H | C ₄₂ H ₇₁ O ₂ P | | B 32.4 | B 0 | 0.09 | | 61.93 | pubche... | B Method: Progenesis MetaScope. Da |
| 123066663 | PA(19.1/9Z)/20.5/5Z,8Z,11Z | M-H | C ₄₂ H ₇₁ O ₂ P | | B 32.4 | B 0 | 0.09 | | 61.93 | pubche... | B Method: Progenesis MetaScope. Da |
| 123066465 | PA(17.2/9Z,12Z)/22.4/7Z,10Z | M-H | C ₄₂ H ₇₁ O ₂ P | | B 32.4 | B 0 | 0.09 | | 61.93 | pubche... | B Method: Progenesis MetaScope. Da |
| 123066957 | PA(22.4/7Z,10Z,13Z,16Z)/1Z | M-H | C ₄₂ H ₇₁ O ₂ P | | B 32.4 | B 0 | 0.09 | | 61.93 | pubche... | B Method: Progenesis MetaScope. Da |
| 123066984 | PA(22.6/4Z,7Z,10Z,13Z,16Z) | M-H | C ₄₂ H ₇₁ O ₂ P | | B 32.4 | B 0 | 0.09 | | 61.93 | pubche... | B Method: Progenesis MetaScope. Da |
| 123066825 | PA(20.5/5Z,8Z,11Z,14Z,17Z) | M-H | C ₄₂ H ₇₁ O ₂ P | | B 32.4 | B 0 | 0.09 | | 61.93 | pubche... | B Method: Progenesis MetaScope. Da |
| GPA(17.1/22.5) | GPA(17.1/22.5) | M-H | C ₄₂ H ₇₁ O ₂ P | | A 32.4 | A 0 | 0.09 | | 61.93 | nonlinea... | A Method: LipidBlast. Precursor tolera |
| GPA(22.4/17.2) | GPA(22.4/17.2) | M-H | C ₄₂ H ₇₁ O ₂ P | | A 32.4 | A 0 | 0.09 | | 61.93 | nonlinea... | A Method: LipidBlast. Precursor tolera |
| GPA(22.5/17.1) | GPA(22.5/17.1) | M-H | C ₄₂ H ₇₁ O ₂ P | | A 32.4 | A 0 | 0.09 | | 61.93 | nonlinea... | A Method: LipidBlast. Precursor tolera |
| GPA(17.2/22.4) | GPA(17.2/22.4) | M-H | C ₄₂ H ₇₁ O ₂ P | | A 32.4 | A 0 | 0.09 | | 61.93 | nonlinea... | A Method: LipidBlast. Precursor tolera |
| GPA(22.6/17.0) | GPA(22.6/17.0) | M-H | C ₄₂ H ₇₁ O ₂ P | | A 32.4 | A 0 | 0.09 | | 61.93 | nonlinea... | A Method: LipidBlast. Precursor tolera |
| GPA(17.0/22.6) | GPA(17.0/22.6) | M-H | C ₄₂ H ₇₁ O ₂ P | | A 32.4 | A 0 | 0.09 | | 61.93 | nonlinea... | A Method: LipidBlast. Precursor tolera |

Figure 32: The LipidBlast and LipidMaps MetaScope search results are automatically differentiated in the results table, where a compound has hits from both.

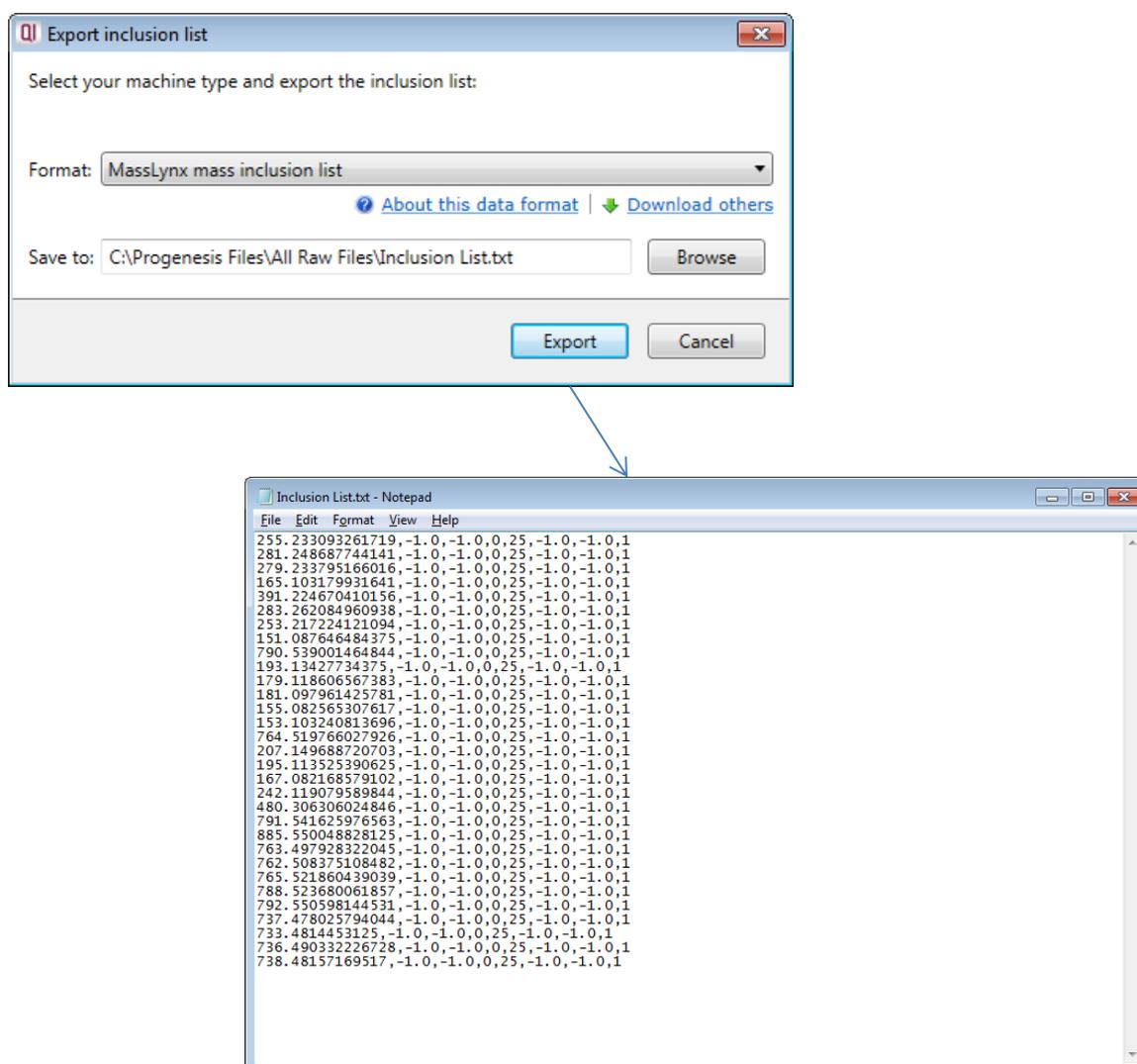


Figure 33: The Export inclusion list feature allows you to generate a targeted list for DDA analysis to improve your identifications.